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THE PRODUCTION OF NON TOXIC CASTOR BEAN MEAL FREE OF ALLERGEN*

Prepared by the
FOOD PROTEIN RESEARCH AND DEVELOPMENT CENTER**

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**Texas Engineering Experiment Station, Texas A&M University System, College Station, Texas, United States of America, under the leadership of Dr. Khee Choon Rhee.

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PREFACE

This contract research entitled "The Development of a Castormeal Detoxification and Deallergenation Technology, Phase III Operations" was initiated on June 1, 1985, as a follow-up of the earlier study on the same subject entitled "Phase II: The Definition and Specifications of a Suitable Technology for Application in Industry." It was carried out under the project "Development of a castorbean detoxification technology and the setting-up of a demonstration plant in a developing country" (US/GLO/77/033), which was to terminate on 31 May 1986, but extended to 31 August 1986 to complete the remaining work and analysis of the data.

At one time or another, the following individuals or firms were involved in various aspects of the research for its successful completion.

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I. INTRODUCTION

Because of its high and unique oil content, castorseed (Ricinus communis L.) has a variety of potential uses. The residue of an oilseed after the oil has been expelled is called pomace or meal. Economics of most oilseed processing plants are therefore based on two valuable major products, oil and meal. However, contrary to high usefulness of the castor oil, castor meal at best can only be used as a fertilizer, far below its potential value as a source of feed protein and carbohydrate, because of several harmful substances it contains. This fact affects industrial castorseed processing economics very unfavorably.

A poisonous heat-labile protein called ricin, a toxic alkaloid component called ricinine, and a powerful and very stable allergen known as CB-1A (Gardner et al., 1960) are the risks of major concern in castorseed industry. Ricin is easily destroyed by heat and is even usually detoxified during desolventization. The amount of ricinine in castorseed is very small relative to toxicity and it presents no particular problem for feed uses as long as moderate levels are fed (Mottola et al., 1968). However, the principal castorseed allergen retains its immune precipitating and allergenic properties even after heating for 1 hr at 110°C at pH 5.9.

The allergen content of several varieties of castor meals ranged 6.1-9.0%, while commercial castor meals contained 0.092-4.2%, equivalent to about 1 to 55% of the allergen content of solvent extracted raw seeds (Coulson et al., 1960). It indicates the effectiveness of industrial processing in reducing the potency of allergen by modern milling practices. But, the sure way to effectively control the allergen problem is the development and implementation of a practical detoxification and deallergenation technology available for industrial application.

The previously reported trials to destroy allergen by chemical and/or physical methods indicated that reactions were too drastic to destroy not only the targeting material, castor seed allergen, but also the essential amino acids needed for animals. Furthermore, no practical scaled-up evaluation of the experimental treatments and measuring the nutritive value and acceptability of products as animal feeds have been undertaken.

A technologically and economically sound detoxification and deallergenation method, when appropriately applied, will substantially increase the commercial value of castor meal by permitting it to be traded as a protein feed ingredient instead of only a cheap fertilizer at present. To achieve this goal it will be necessary to carry out a series of in-depth biochemical evaluation of the treatment as well as a thorough safety, acceptability and nutritional evaluation of the final product through feeding tests of several species of animals.

II. OBJECTIVES

The ultimate goal of the project is to develop a practical and scientifically-sound detoxification and deallergenation technology which can be applied to industrial production of safe castor meal for use as a protein animal feed component. To achieve this goal, the following specific objectives were outlined in the contract.

1) Make available up to fifty tons of commercial solvent extracted castor meal as the raw material for extruder testing operations and determine its quality criteria and other relevant analytical data.
2) Based on the results obtained from the pilot plant operations, carry out extruder test operations with the aim to scale-up the detoxification and deallergenation technology to the industrial scale and to reproduce and prove its effectiveness at the minimum of three different scales to be determined.
3) During the scaled-up extruder operations carry out current product quality control work by using the analytical control methods developed during the pilot plant operations and thereby test and prove their efficiency and reliability.
4) On each of the scaled-up extruder test operations (minimum three) determine and specify the technological parameters and other relevant data as the basis for the final defination of the overall castormeal detoxification and deallergenation technology to be developed and documented.
5) Collect the processed (extruded) detoxified and deallergenized castormeal as the final product and make it available for animal feeding tests to follow.

6) Arrange the professional conduction of comparative animal feeding tests on rats, poultry and pigs by using the extruded (detoxified and deallergenized) castormeal as a protein feed component. Describe in detail the arrangements made, procedures and results obtained over a suitable feeding test period.

7) Carry out special laboratory scale/pilot plant work on the detoxification and deallergenation of prepressed cakes instead of solvent extracted meal with the aim to possibly apply the new detoxification and deallergenation process prior to the solvent extraction operations and not as a tail-end activity as originally envisaged.

8) In case the detoxification and deallergenation process should successfully be applicable on prepressed cakes, further develop this technology for alternative industrial application whenever required and include this process in the final documentation.

9) Carry out experimental animal feeding tests on rats and other animals if applicable in order to specifically test the effect the residual oil content of prepressed detoxified and deallergenized cakes may have on the animals digestive system.

10) Carry out especially designed in-depth studies in view of creating a better insight view and understanding of the nature of castorbean toxins and allergens and their actions and reactions leading to detoxification and deallergenation results.

11) Based on the results obtained from the in-depth study work of castorbean toxins and allergens as mentioned under 10) above further develop and possibly simplify the hemaglutinin reaction and immuno diffusion method as industrially applicable product quality control methods.

12) Finally describe both product quality control methods the way they have to be prepared, applied and evaluated in relevant laboratories normally attached to commercial castorbean processing factories (Oil Mills).

13) Finally describe and suitably outline by means of relevant diagram and/or otherwise the new castormeal detoxification and deallergenation technology and its techno-economic parameters in connection with the examples of a 25 ton/24 hours and a 50 ton/24 hours castorbean processing plant consisting of prepressing and solvent extraction (hexane) operations. The definition and description of the new technology - if applicable - is to be alternatively made as front-end (pre-pressed cakes) or tail-end (extracted meal) operations.

14) Be prepared to review and study a castorbean processing factory to be nominated by UNIDO and as the result outline, describe and specify the castormeal detoxification and deallergenation technology for demonstration in this particular plant. This item of work will only be applicable on UNIDO's special notice and will be made a point of special organization and financial arrangements exclusive of this contract.

III. SCOPE OF WORK

The scope of this project is limited to the fourteen objectives listed in the "Objectives", which include limited scale animal feeding tests for nutritional and toxicological evaluation of products. The degree of detoxification and deallergenation as well as nutritional and toxicological properties of products were therefore assessed by evaluating the combined results from chemical, immunological and animal tests. The animal feeding studies were conducted according to the standard methods and procedures established by U.S. government agencies for such tests; however UNIDO is advised of the fact that the limited scale nutritional and toxicological tests through rat, poultry and swine feeding studies are preliminary in nature, and may not be comprehensive enough to meet all requirements established by national and international regulatory agencies for such purposes.

Also, this project is not intended to produce a final detailed design for a detoxified and deallergenated castormeal production plant. Although much of the information generated from the successful execution of the project would be used for such design purposes, additional information from expert plant design engineers would be needed to ensure the construction of an economically viable full-scale production plant.

IV. SELECTIVE LITERATURE REVIEW

The castor plant Ricinus communis L. (Euphorbiaceae) originated from Asia and Africa, but is now growing even in Europe and America. It is usually an arboreus plant 1-5 m high, but in tropical regions the plant grows to a high tree which is characterized by its rapid growth and sudden wilting (miracle tree) (Olsnes and Phil, 1977). The ancient Egyptians cultivated castor bean for oil. For a century, the extracted oil has been valued as a safe and very efficient cathartic (Mottola et al., 1970). The castor seed contains about the half content of oil, of which ricinoleic acid occupies more than 93%. Today, special characteristics of the oil such as the high viscosity, resistance to heat and pressure, low freezing point and ability to form a waxy substance upon chemical treatment (Lehrer et al., 1980), the oil and its derivatives possess a variety of usage in paints, varnishes, surfactants, cosmetics and pharmaceuticals (Mottola et al., 1970). On the contrary, the pomace has only been used as a fertilizer and even this usage has some hazard because of the potent allergens it contains (Spies et al., 1962).

IV.1. Castor Bean Allergen

IV.1.1. *General Properties*

An allergen, as defined by Spies (1974), is "an ordinary hundreds of substances present in the diet or environment capable of producing such diseases as asthma, hay fever, eczema and gastrointestinal upsets upon contact with a previously sensitized person". Allergy is "the body response to an allergen-antibody reaction which triggers the release of chemical mediators of hypersensitivity, namely, histamine, serotonin and acetylcholine, as well as larger compounds, slow reducing substance (SRS), and the plasma kinins (Austen, 1965). Antigen is defined as "a molecular species capable of inducing an immune response and of reacting specifically with the products (antibody, sensitized cells) manufactured as a consequence of the immune response". The ability of a material to react with the products of immune response is referred to antigenecity, and therefore such a material is an antigen (Sell, 1980).

Castor bean allergen, a proteinaceous fraction of the castor seed aqueous extract called CB-1A, has been described for its isolation, purification and immunological properties since 1940's. CB-1A was first isolated according to the procedure developed for CS-1A from cottonseed by Spies and Coulson (1943) and was characterized as being insoluble in water, soluble in 25% alcohol and basic lead acetate solution, but is being precipitated by 75% alcohol. It does not precipitate in boiling water and is partially dialyzable.

The yield of allergens in castor meal shows somewhat different values according to the research. This might be reflected mainly by the purity of the obtained allergen, occurrence of other properties and compounds (Youle and Huang, 1978b), and differences in plant variety and its growing areas (Olsnes and Phil, 1973). Spies and Coulson (1943) isolated a nontoxic, highly allergic glycoprotein fraction which corresponds to 1.8% of the defatted meal. Spies et al. (1944) also reported yields of 0.74% and 0.18% dialyzed allergen from domestic and Brazilian castor pomace, respectively. Waller and Negi (1958) reported lower yields than the previous workers, yielding 0.08% and 0.22% purified allergens from commercial and laboratory samples, respectively. Gardner et al. (1960) reported allergen, CB-1A, represented as high as 12.5% of the weight of the isopropyl alcohol-extracted castor pomace, as determined by antigen-dilution precipitin test. Coulson et al. (1960) reported more detailed data from ten different commercial castor pomaces. The allergen content ranged from 6.1% to 9.0%, according to variety and 0.092% to 4.2%, according to pomace processors, respectively. Based on these results, the authors suggested the discouraging prospect for the development of an allergen-free castorbean by breeding, as well as encouraging the possibility of reducing the allergen content of castor pomace by employing suitable processing devices. The reported nitrogen values of the allergen ranged from 16.8% (Waller and Negi, 1968) to 18.9% (Coulson et al., 1950).

CB-1A contains no ricin and is virtually nontoxic, but was thought to be a glycoprotein fraction of highly allergic (Spies and Coulson, 1943; Spies et al., 1944; Spies et al., 1951). The castorbean allergen was described as a group of low molecular weight microheterogenous proteins having similar chemical properties (Spies and Coulson, 1964; Spies, 1967) or a mixture of proteins and polysaccharidic proteins (Spies and Barron, 1966), classified as natural proteoses - a subclass of native proteins (Spies, 1974), existing as low molecular weight albumin storage proteins in the matrix of the bodies of the castor bean endosperm (Tully and Beevers, 1976; Youle and Huang, 1976, 1978a, 1978b).

On the basis of molecular weight, only the 2S storage protein of seeds is compared well with CB-1A fraction physio-chemically (Youle and Huang, 1978b). Two resolved groups of protein bodies, the high molecular weight proteins representing 11S globulins, 4-6 albumins and their associated subunits and the low molecular ones of 2S albumins, were detected. Among them, only albuminous 2S fraction (around 11,000 daltons) showed the similar electrophoretic patterns, antigenic relationship, thermal stability and solubility, and amino acid composition with CB-1A.

Amino acid determination of the allergen showed relatively high arginine, cystine and glutamic acid contents and no tryptophan (Spies and Coulson, 1943; Spies et al., 1951). The low molecular weight compounds presented in the castor seed allergen fraction were thought to be present as complexes consisting of only guanido compounds, or agamatine, arginine and two other unknown guanido derivatives (Smola and Robinson, 1969).

IV.1.2. *Immunological Characterization*

Castor bean allergen is known to be the most heat stable proteins ever known. Boiling the allergen at 100°C for 1 hr did not destroy or diminish the activity of the native antigenic structure (Coulson et al., 1950) and the immune precipitating and allergenic properties are also retained even after heating at 100°C in alkaline solutions (Spies et al., 1960). Recently, Lehrer et al. (1981) suggested that both heat-stable smaller molecular weight and heat-labile, larger molecular weight allergens were present in castorbean extracts. Both allergens were immunochemically distinct and had different isoelectric points, but thought to be chemically relevant since coffee workers sensitized to castor allergen had IgE antibodies to both allergens. However, heat-stable allergen was chemically significant because response to the heat-stable allergen was stronger than to the heat-labile allergen.

It is a generally recognized concept that Fraction CB-1A contains the principal allergic specificities and are immunologically distinct from other allergens and antigens of different source materials (Spies and Coulson, 1964). The Fraction CB-1A was reported as a complex mixture of polysaccharidic glycoproteins and proteins having a major common antigenic specificity (Spies and Coulson, 1964). The principal components of CB-1A have been identified using various separation techniques along with the test of antigenic and allergenic specificity of each fraction. The paper strip electrophoresis of a water-soluble, heat-stable protein component of castormeal in phosphate buffer at pH 8.0 (ionic strength 0.05) gave a sharp resolution into six distinct and possibly two more components. Each major component was found to be antigenic by passive cutaneous anaphylaxis (PCA) in guinea pigs which had been sensitized with rabbit antiserum to crude castor bean protein, among which five bands were shown to be allergic to humans (Layton et al., 1961).

The existence of six antigenic or allergenic components which possibly had various antigenic specificities in CB-1A was also confirmed with the use of a

DEAE-cellulose column chromatography for separation and the Schultz-Dale technique for testing antigenic specificity (Layton et al., 1961). Spies and Coulson (1964) obtained ten subfractions from CB-1A by combination of dialysis and ion exchange chromatography. The important subfractions were further characterized by cellulose acetate electrophoresis and their antigenic subfractions were studied employing Schultz-Dale technique, gel diffusion, immunoelectrophoresis and antibody absorption technique. Results showed that two chemically distinct components of Fraction CB-1A had similar antigenic specificities and at least one and possibly two antigenic specificities distinct from the major common specificities were existing. Spies and Barron (1966) found that four chemically distinct principal components of CB-1A contained identical antigen specificity. A cellulose acetate electrophoresis fractionation of CB-1A, a quantitative electrophotometric analysis of CB-1A Ponceau S complex and the identification of antigenic specificity by gel diffusion technique showed that four principal antigens which occupy 85% of the total complex and the principal minor antigen were completely separable and both were allergenic. Spies (1967) later separated ten subfractions of CB-1A and a minimum of eight antigens by gel diffusion analysis from electrophoretic bands.

Fraction CB-1A could be completely separated into subfractions because they are chemically distinct (i.e., different carbohydrate content of the polysaccharidic-protein complex), but a major common or identical specificity is shared by each subfraction. Antigenically, Fraction CB-1A is heterogenous and is accompanied by one, two or more of minor specificities (Coulson et al., 1949). It is also known that each distinct antigen may function as a distinct allergen, but the principal allergen governs the antigenic specificity over all subfractions of CB-1A. At least one or more allergens, distinct from CB-1A, was observed in the castormeal (Spies and Coulson, 1964).

It is a generally accepted concept that "1A" (including other natural proteoses) contains multiple components which show similar allergenic and antigenic specificities, and it is the active protein or combination of protein and various proportions of polysaccharidic carbohydrate that determines the allergenic and antigenic specificity (Spies and Coulson, 1964). It was reported that the allergenic and anaphylactogenic properties of CB-1A and its purified, carbohydrate-free subfraction, namely, CB-65A which was prepared by a series of procedures involving chromatography, electrophoresis and solvent fractionation, were directly related to the carbohydrate-free passive transfer reaction of the protein (Spies et al., 1944; Coulson et al., 1946). It is also reported that polysaccharidic portion of CS-1A and CB-1A enhanced the degree of sensitizing in guinea pigs, but their specificities or precipitability did not change on a same nitrogen basis (Coulson et al., 1949; Coulson et al., 1950; Morris et al., 1965). When the component of CB-65A was polyacrylamide-gel (7.5%) electrophoresed, the fraction was resolved into four bands whose immunological specificity was closely similar, if not identical. It was thus concluded that any existing structural differences were too slight to impart immunological distinctiveness among proteins.

IV.1.3. *Allergenicity of CB-1A*

CB-1A has been known as a strong allergen. he Fraction CB-1A from castor seed is capable of precipitating when given to rabbits (Coulson et al., 1946), but it is not a toxin (Spies and Coulson, 1943; Spies et al., 1960; Spies et al., 1962). The minimum dose which could cause sensitivity and anaphylactic shock (SD_{50}) in guinea pigs ranged from 8.4 µg (Spies and Coulson, 1943) to 12 ± 2 µg (140 ± 40 µg nitrogen for CB-65A) (Coulson et al, 1950) on nitrogen basis. The median lethal dose (LD_{50}) of CB-1A in guinea pigs, previously sensitized with 1 mg of the antigen, was 0.43 ± 0.06 µg nitrogen (Coulson et al., 1950). Human patients who were sensitive to the allergenic fraction showed cutaneous reaction when they received a $1{:}10^6$ dilution (Spies and Coulson, 1943).

A cutaneous phenomenon like urticarria occurring on the exposed skin of the castor seed sensitive person is the most common disease symptom (Panzani and Layton, 1963). The area of body where clinical symptoms generally occur are nasal, pharyngeal and respiratory system, mucous, and oscular conjunctives (Layton et al., 1961; Lehrer et al., 1981). In the respiratory system, it appears as an allergic type of rhinopharyngitis, as well as presenting general symptoms such as chronic headache and fever. In the eye, it produces conjunctivitis (Layton et al., 1962; Lehrer et al., 1980).

IV.1.4. *Other Evidences of Castor Bean Allergen*

Many industrial workers who process castor beans for oil or handle fertilizer containing castor meal become sensitized to the allergens (Coulson et al., 1950; Small, 1952; Panzani, 1957). It is also known that light dursty powder of castor pomace can easily be spread widely by winds and cause severe allergy in the area where it is produced or handled like solvent extraction factory or shipping area (Aspen et al., 1967).

There are many reported cases of allergenic sensitivity in coffee industry relating to castor bean allergen. Figley and Rawling (1950) first recognized that the occupational allergic disease of coffee workers could be caused by castor bean allergen contamination. This clinical evidence was well supported by the identification of castor bean allergen in green coffee (Coulson et al., 1950). Bernton (1973) reported a case of sensitivity to castor seed of a coffee industry worker who showed intermittent asthmatic symptoms which were particularly severe when he worked in the mill. Karr et al. (1978) reported the case of coffee worker's asthma. Serum IgE antibodies specific for etiologic green coffee bean and castor bean antigen were demonstrated by the serum radioallergosorbent (RAST) test (Ceska and Lundkvist, 1972) from the coffee workers with occupational allergic disease. The antigen present in certain industrial dust and sack sample which were capable of producing asthma in sensitized subjects were distinct and unrelated to chlorogenic acid (Layton et al., 1963, 1965a, 1965b, 1968) which had been reported as a major allergen present in both coffee and castor bean (Freedman et al., 1961).

The respiratory allergy to ricinus seed (castor bean) can also cause cross-reactivity with other plants of the Euphorbiaceae (spurge) family, such as rubber tree (Hevea brasiliensis), tung (Aleurites cordata), cassava (Manihot ultissma) - the source of tapioca and a potential new oilseed crop (Euphorbia lagascae) (Layton et al., 1970). Accordingly, the authors recommended that patients should be admonished not to come into contact with species closely related to the species primarily responsible for their allergy symptoms.

The castor meal allergens can be transmitted to man through the feed. According to Layton (1977), catfish could be fed with a ration with up to 50% of castormeal without harming the fish, but the allergenic substances reached the muscles in sufficient quantity to produce allergy to those who were sensitive to castor seed. The author suggested to have the fish, which had been fed with castormeal, several days without this feed just before harvest.

Although castor oil has been known virtually free of potent castor bean allergen (Panzani and Layton, 1963), the presence of castor bean allergens in castor wax was reported by Lehrer et al. (1980). The results were demonstrated by PCA reactions in rats, a positive direct RAST for reaginic castor allergens and positive skin reactions in sensitized individuals. This is of concern because wax is extensively used in a variety of industrial and medical products. However, the allergens in the wax were of much lower potency than those in the bean and were not detectable in a deodorant product using castor wax.

IV.1.5. *Deallergenation Approaches*

Since the risk of extremely potent allergen in castor beans and castor pomace has been a deep concern to castor industries, growers, fertilizer handlers, oil processors, traders, public health officials and even the residents in the vicinity of such installment, various exploratory experiments on the deallergenation, including detoxification of heat-labile ricin, have been proposed by many researchers. The complete destruction of the allergens from castormeal would substantially enhance the current value of the pomace by 1.7 times or more (Mottola et al., 1971) by permitting it to be traded as a protein feed ingredient instead of only a fertilizer at the present.

Most of the reported trials for the deallergenation of the castor meal largely include: cooking of the castor seed cakes or meals under various conditions of moisture, pH, temperature and steam pressure; treatment of chemicals such as sodium hydroxide, sodium hypochlorite, hydrochloric acid, sodium chloride, ammonium sulfate, urea, potassium permanganate, calcium hydroxide and lime with steam; biological treatments including aerobic fermentation and enzymatic digestion of the extracts.

Gardner et al. (1960) reported the results of a series of probing experiments of flaked castor bean meats and pomaces for the detoxification and deallergenation, providing possible ways for further research. The treatments were concentrated into four categories:

1) Alkaline treatment. The flaked castor bean meat was premoistened to about 16% by the addition of water with or without chemicals. The moist cooking at 100-102°C (212-215°F) for 12-15 min with 1% NaOH was effective in reducing the allergen content by 97 to 98.4% from the original amount, as compared to the 50-75% reduction for the control (nonalkali treatment). Cooking with 2% NaOH at 20 psi pressure yielded a complete destruction of the allergen. The use of potassium hydroxide and calcium hydroxide in moist-cooking at 20 psi was less effective than that of sodium hydroxide in reducing the allergen. Regardless of allergen content, ricin components were completely inactivated.

2) Ammonia treatment. Ammonia was used to further reduce the CB-1A content of pomace which had been partially deallergenized to about 0.8% CB-1A content by alkali-cooking of raw flakes. No apparent reduction was made over control, however, the reduction down to 0.2% CB-1A was indicated when the treated pomace was hermetically stored for 45 days.

3) Dry-heat treatment. A simple heating at 207°C (405°F) for 125 min for the partially deallergenized pomace could completely inactivate the allergen. However, a considerable heat damage of the product was also accompanied.

4) Chemical and biological treatment. The best results were obtained with formaldehyde (HCHO). The complete deallergenation was available with the following treatments: 3% HCHO and 1% NaOH, 3% HCHO and 0.9% HCl, and 10% HCHO and 2% NaOH, respectively. Treatment of alkaline pomace with 1% NaOCl (sodium hypochlorite) or 2% HCl did not further reduce the allergen content.

Trypsin digestion in an aqueous alkali medium or partially deallergenized pomace prepared from alkali-cooked flaked meats could get the 98.4% reduction of the allergen.

Among all trials, the five most promising treatments listed with the indicated possible reductions measured by antigen-dilution precipitin test and Schultz-Dale test (in parenthesis) were as follows: dry heating of pomace to 205°C (401°F) (100%, 100%), moist-cooking of flaked meats with 2% NaOH and 10% HCHO (100%, 99.9%), moist-cooking with 0.9% HCl and 3% HCHO (100%, 99.9%), moist-cooking with 2% NaOH at 20 psi pressure (100%, 100%) and moist-cooking with 1% NaOH (98.4%, unavailable).

Mottola et al. (1971) reported a pilot-plant steam process for castor meal antigen deactivation. The relative effect of various levels of low-pressure steam and time was evaluated using more accurate intradermal technique (Mottola et al., 1970), PCA test and potency evaluation, as an antigen deactivation indicator, instead of intravenous biological assay technique which had been used in the previous report (Mottola et al., 1967). By changing the antigen evaluation method, less severe treatment for the antigen destruction was suggested as follows. Twelve combinations of 10, 20, 40 and 80 psi steam pressure and 15, 30 and 60 min heating time showed a pronounced effect on the residual allergen potency. It also gave an evidence for significant time and pressure x log dose interaction. The overall biological response levels decreased as dilution ratio and treating time increased although a temporary increase of ID potency ratio was noted between steam pressures from 10 to 30 psi, possibly due to the increased antigen extraction than destruction at those ranges. A significant loss of lysine occurred through heat treatment, while the remaining level of arginine was still as good as those found in soybean meal. The mild heat-treatment of 10 psi steam pressure for 60 min was recommended by the authors for the considerable allergen destruction and the best preservation of the essential amino acid, lysine.

A series of reports on the process to deactivate the castor meal antigen has been made through the leadership of Mottola and his co-workers. The variables of ammonia process affecting antigenecity responses of castor meal were reported by Mottola et al. (1972a). The ammonia concentration, process temperature, quantity of liquid in the slurry and process time were evaluated against antigenecity with abdominal intradermal injection upon passively sensitized guinea pigs. Temperature itself was proven to be very critical over other parameters while 6M NH_4OH was most effective over 1, 2, 4 and 15 mole concentration values. The biological response with guinea pigs (Mottola et al., 1970) indicated positive responses up to 1:100 dilution at 80°C treatment, while both 20°C and 50°C treatment showed positive reactions up to 1:1000 dilutions.

The proposed processing conditions to prepare sufficient quantity of deactivated meal for poultry and cattle feeding trials consisted of liquid (6M NH_4OH solution) to solid ratio of 1:4 and process temperature of 80°C for 45 min in the Patterson vessel. The degree of antigen destruction was reportedly comparable to that obtained with high pressure steam.

Mottola et al. (1972b) also described a pilot plant processing using lime to deactivate the castor meal antigen. In this report, the authors re-evaluated the previously reported result (Mottola et al, 1968) by introducing a different assay method for antigenecity. Previously, the antigen challenge was indicated by the bluing depth of Evans blue dye (Layton et al., 1961) of the skin via the cephalic vein of albino guinea pigs, while, in the later, a more sensitive and less variable intradermal injection at the site previously sensitized by anticastor serum. The optimal deallergenation condition proposed was the treatment with 4% $Ca(OH)_2$ at 120°C for 15 min in a steam-jacketed Patterson reactor. By introducing more accurate antigen analysis method, Mottola et al. (1972b) could suggest much milder optimum conditions than those of the previous work where the treatment with 8% lime at 140°C for 60 min in the same reactor (Mottola et al., 1968) was given. The amino acid profiles showed the lime processed products had the most detrimental effects on cystine, hydroxy amino acids like serine and threonine, and methionine contents, followed by steam processed product (Mottola et al., 1971) and ammonia processed one (Mottola et al., 1972a). Because of the loss of the several essential amino acids, Mottola et al. (1972b) precluded the use of deallergenized meal in rations for poultry and swine, but recommended to use as ruminant rations, since cattle and sheep do not have the essential amino acid requirement of monogastric animals.

Spies et al. (1962) also determined the conditions of time, temperature and pH for the deactivation of ricin and allergen with lime. The authors also correlated the immune-precipitating and passive-transfer methods according to the reagin-neutralizing properties of guinea pigs in relation to allergen destruction. The castor meal was heated with 0-16% (w/w) $Ca(OH)_2$ for 1 hr at 60, 80 and 100°C, respectively. At 60°C, no complete destruction was obtained regardless of chemical concentrations while 80° and 100°C treatments showed the negative immune-precipitating property at 8% concentrations of calcium hydroxide. Under the fixed temperature (100°C) and calcium hydroxide concentration (8%), the length of heating-time required for the complete destruction of immune precipitating property of allergen was noted as 32 min. With the same temperature (100°C) and heating time (32 min) conditions the calcium hydroxide concentration required for the negative skin reactivity was shown as 16%. In a series of immune-precipitating and reagin-neutralizing testings at different heating temperature, time and calcium hydroxide concentrations upon the dialyzed CB-1A, it was observed that pH of the slurry was affecting the accuracy of the testing results. At pH 5.9 to 8.7, the immune-precipitating property was more stable to heat than the reagin-neutralizing property, but at pH 10.8 to 11.9, the reverse was observed.

Other series of deallergenation trials using sodium chloride, potassium permanganate, ammonium sulfate, urea and aerobic fermentation on castor meals showed the allergen destruction ratio less than 75% (Gardner et al., 1960), a number which is too low to be considered for practical applications.

IV.2. Ricin

Besides the allergenic fraction, the castor bean pomace also contains a highly toxic albumin, ricin, and a mildly toxic alkaloid, ricinine (Waller and Negi, 1958). The ricinine is present in a very small amount and because it is not seriously toxic, it is not considered particularly detrimental (Gardner et al., 1960).

IV.2.1. *General Properties*

A purified ricin was first prepared by Osborne et al. (1905) by saturating the slurry with sodium chloride, followed by a series of ammonium sulfate precipitation. The final preparation showed a strong precipitating activity and lost its activity completely when heated. Finally, it was concluded that ricin was a protein of high molecular weight, represented by 1.0-1.5% of the defatted meal (Waller and Negi, 1958). Later, Kabat et al. (1947) extracted a highly toxic fraction from the castor seed presscake using a slightly modified method of Osborne et al. (1905). The "Ricin B_1" thus obtained showed a molecular weight between 77,000 and 85,000 and was homogenous electrophoretically, ultracentrifugally and immunochemically. Kunitz and McDonald (1948) identified the "Crystalline ricin" as a globulin type protein with an isoelectric point at pH 5.4-5.5 and molecular weight of 36,000. Its toxicity was higher, but the hemagglutinating activity was far lower than that of Ricin B_1.

Funatsu (1972) were the first to separate a nonagglutinating toxic component from a nontoxic agglutinin. The toxic component is now generally called ricin and is composed of two polypeptide chains held together by disulfide bonds (Olsnes et al., 1974a; Funatsu and Funatsu, 1977).

"A-chain" was found to inhibit protein synthesis in a cell-free system of rabbit reticulocytes, while "B-chain" functions as a carrier moiety, which serves to anchor the toxin to the cell surface, a binding that probably involves galactose-containing receptor sites (Olsnes and Phil, 1973; Olsnes et al., 1974a, 1974b). The binding of the toxin causes an increase in membrane fluidity and the A-chain (or possibly the whole toxin) is transported into the cytoplasm, where it exerts its toxic effects.

A further purified ricin, "Ricin D" (Funatsu, 1972), was found to be a glycoprotein containing 5.6% mannose and about 2% glucosamine. The N-terminal amino acids of Ricin D were alanine and isoleucine while C-terminal amino acids were phenylalanine and serine (Ishiguro et al., 1971). Funatsu et al. (1977) described that tyrosine and lysine residues in each chain of Ricin D were involved in the toxic action.

The high toxicity of castor bean which affects human and domestic animals have been known since ancient times. Stillmark at Kobert's laboratory in Estonia was the first one who carried out the extensive study of the castor bean toxin and suggested the name ricin in the late nineteenth century (Olsnes and Phil., 1977). Later, ricin has been recognized as a kind of phytotoxins which includes highly toxic compounds such as abrin, crotin, circin and rubin. All these compounds are antigenic, thermobile and possess hemagglutinating properties (Kabat et al., 1947).

Ricin is usually determined by biochemical and biological assay methods using its hemagglutinating and toxic properties. A chemical analysis method is not practical because it is associated with another protein materials just like other plant hemagglutinins. A detection of lectins in plant extracts is still performed mostly by a serial dilution technique with visual estimation of the end point (Jaffe, 1969). A hemaglutination test using the agglutination of blood red corpuscles, suggested by Gardner et al. (1960), is known to be very practical and relatively accurate for the qualitative and quantitative estimation of the ricin. The modified method of Gardner et al. (1960) and Bukhatchenko (1973) and other trials have been proposed for the increased accuracy and time-saving (Jaffe, 1969; Clarke, 1973; Kaneko et al., 1975; Liener, 1976), while some of the proposed methods have been critically evaluated by Burger (1974).

IV.2.2. *Toxicity of Ricin*

The toxicity of ricin varies with animal species. On a same weight basis, horse and guinea pig are highly sensitive to ricin while birds are more resistant than mammals (Jones, 1947). A purified ricin prepared by Kabat et al. (1947) was proven to be fatal to a rat within 24 hr by 7.8 gamma/100 g weight. Waller and Negi (1958) reported that the lowest tested dose killed a 100 g rat in 48 hr was 9.5 gamma. The MLD is about 0.001 μg/g rat, which means it is 1,000 times more toxic than other seed lectins. It's toxicity persists even after oral ingestion, accordingly, castormeal should essentially be detoxified to be used as animal feeds (Liener, 1974). The most common findings of the physiologial effects are hemorrhages in the intestine, mesenterim, omentum (Olsnes and Phil, 1977), paralysis of the respiratory and vasomotor system accompanied by common illness symptoms (Jones, 1947). It is also indicated that toxic effect is primarily the result of the inhibition of the protein synthesis (Montanaro et al., 1973, 1975) and, reversely, there has been attempts to use ricin for the treatment of human cancer (Lin et al., 1970).

IV.2.3. *Detoxification Approaches*

Various means and ways have been reported to eliminate the health hazard of ricin from the castor meal without affecting the nutritional value of the residual protein as an animal feed component. It is well known that ricin, contrary to castor bean allergen, loses its characteristic property, agglutinating the red blood corpuscles of the mammals, when it is heated to the boiling point or coagulated by heating to 60-70°C, while dry heat is not effective for the complete destruction of toxic property of the protein (Jones, 1947). Ricin is also affected and detoxified by sodium ricinoleate, potassium permanganate, hydrogen peroxide and halogens (Carmichael, 1929). It is also reported that the normal extraction and desolventization processes of the meal were capable of destroying ricin (Mottola et al., 1968, 1972a, 1972b, Liener, 1974).

Various conditions to detoxify or reduce toxicity have been proposed using one or the combination of the treatments consisting: heating temperature and time (Petrosyan and Ponomorov, 1937; Tangle, 1939; Jaki, 1941; Chiego, 1950); chemicals like halogens and alkalis (Massart and Massart, 1942); acids (LeBrenton and Gregory, 1957); extracting with solvents like ethanol or chloroform (Rudolph 1943); UV rays (Balint, 1973); and microbials like proteolytic enzymes, autolyzed yeast, and autolyzed acetobacter (LeBrenton, 1950) or Clostridium (Darzins, 1960). Among the various activities for the detoxification of ricin, the heat treatment with or without mild chemicals looks most feasible for practical applications. Kodras et al. (1949) suggested the conditions of autoclaving the meal without water addition for 15 min at 125°C. According to Jenkins (1963), autoclaving castor meal for 1 hr at 15 lb/in^2 could reduce to 1/2000 of the original toxicity, thus providing no significant problems to the feeding rats for 4 weeks in a 23.2% substitution with the treated meal although the growth and feed conversion were less than in control diets. Gardner et al. (1960) could detoxify ricin completely only with mild moist heating either with or without added alkali. Similar result was also reported by Spies et al. (1962) using calcium hydroxide. Funatsu et al. (1965) could detoxify ricin by autoclaving castor meal at 125°C at 2 atm pressure for 5 min in the presence of excess water. A series of conditions reported by Mottola et al. (1967, 1968, 1971, 1972a, 1972b) for the deallergenation of the castormeal were also effective for the destruction of ricin simultaneously. It is a generally accepted concept that toxin itself is of less concern comparing to allergen of the castor meal because ricin is ordinarily destroyed during desolventization of the castor bean flakes and too little ricinine is existing to present serious difficulties (Mottola et al., 1968).

V. EXPERIMENTAL

V.1. Materials and Methods

V.1.1. *Raw Materials*

Thirty-three tons of prepressed, hexane defatted castor meal was purchased from Deutsch Rizinus Oelfabrik Baley GMBH & Co. in Uerdingen, West Germany, and shipped in 50 kg paper bags to College Station in June, 1985. Additionally, 4 tons of castor seeds were also purchased from Bothwell Enterprises in Plainview, Texas. Information on the variety of the seeds was not available.

V.1.2. *Chemicals*

All chemicals used in the experiments, except hexane and ethyl alcohol, were of reagent grade. Technical grade hexane and ethyl alcohol were used since they currently are used in the industry for extraction of oils from various oilseeds.

V.2. Experimental Procedures

V.2.1. *Preparation of CB-1A Antibody*

V.2.1.1. *Preparation of Defatted Castor Meal*

Defatted castor meal was prepared from castor seeds for use in preparation of purified CB-1A. Care was taken to minimize the exposure of all products to extreme temperatures in order not to denature the allergens as much as possible.

Whole castor seeds (25 kg) were cracked using a cracking roll. Hulls then were separated from kernels by air aspiration. The dehulled kernels were flaked to an average thickness of approximately 0.3 mm (0.012 inches), using a flaking roll. The flakes were extracted, using 99% ethyl alcohol, at room temperature for 5 hr with occasional agitation. The flake-to-alcohol ratio was 1:3 (w/v). The extraction was repeated five times, with fresh ethyl alcohol each time, to ensure complete removal of oil. After each extraction, the alcohol was separated from the meal by filtering the slurry through cheesecloth (usually 4-layer thickness). The meal was air-dried at room temperature in a forced-air hood, then stored in a freezer in tightly sealed glass bottles.

V.2.1.2. *Separation and Purification of CB-1A*

The allergen CB-1A was separated from the defatted castormeal and purified according to the procedure outlined by Spies and Coulson (1943) with slight modifications (Figure 1).

V.2.1.3. *Identification of the Purified CB-1A*

A disc gel electrophoresis was performed to identify the typical CB-1A bands. Polyacrylamide gels (7.5%) and Tris buffer (pH 8.6) were used for the test. The details of the disc gel electrophoresis procedures are described later in "Section V.2.6. Biochemical Evaluation of CB-1A".

An animal test was additionally performed with rats (body weight range of 128-154 g) to determine if the purified CB-1A had any allergic effects. Three different solutions, i.e., 0.85% saline solution alone as a control, crude defatted meal extract in 0.85% saline solution, and 0.85% saline solution of the purified CB-1A, were intraperitoneally injected to different rats. Symptoms developed after the injections were observed. The amount of 0.85% saline solution injected was approximately 0.5-0.7 mL, containing 10-15 mg protein.

Subcutaneous skin tests also were performed to determine the allergic effects by applying the purified CB-1A on the skin of the previously sensitized rats.

V.2.1.4. *Preparation of the CB-1A Anti-Serum (Antibody)*

The purified CB-1A was dissolved in the physiological saline solution with the antibody-formation stimulator, "Complete Adjuvant" (Cooper, 1977).

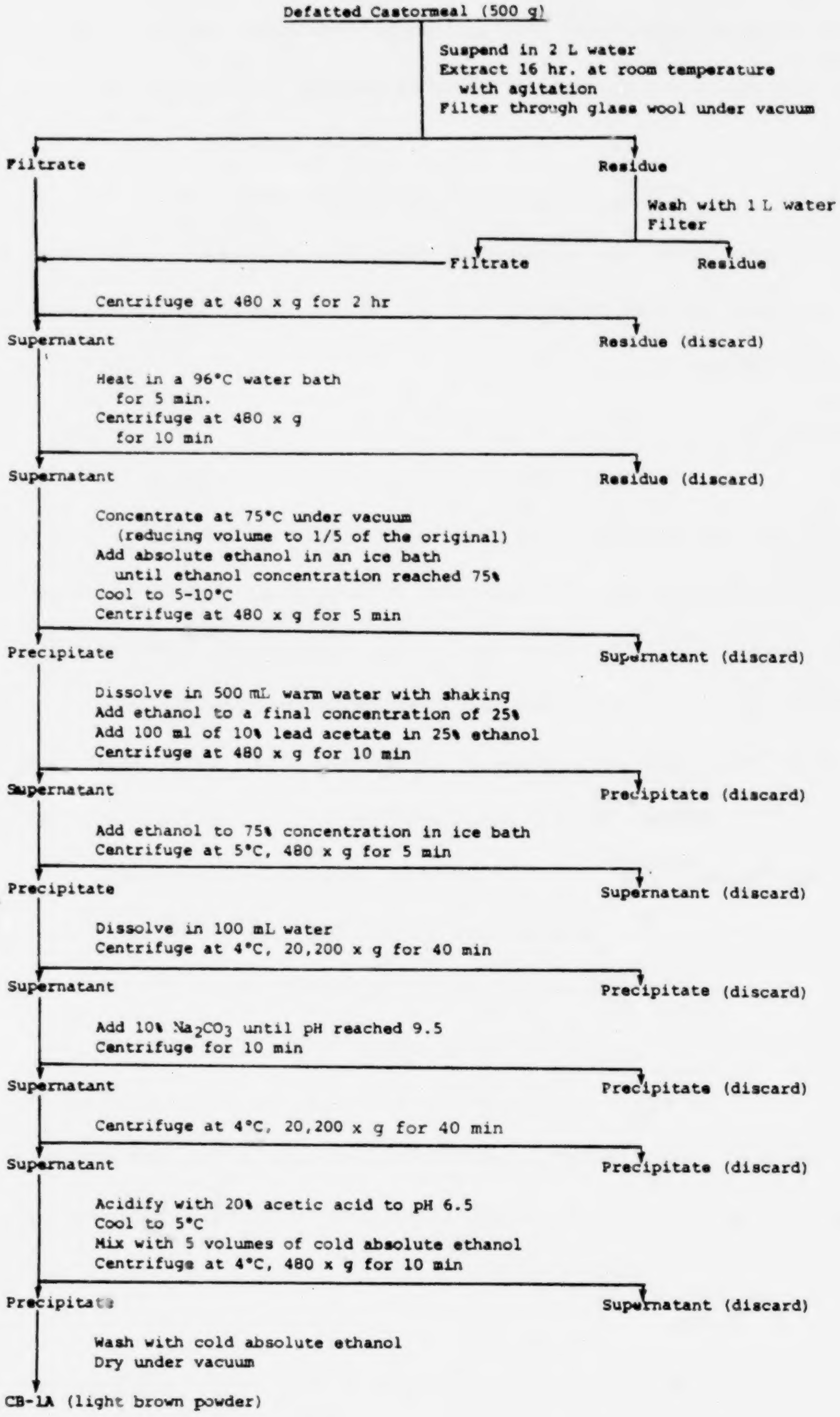

Defatted Castormeal (500 g)
Suspend in 2 L water
Extract 16 hr. at room temperature with agitation
Filter through glass wool under vacuum
Filtrate
Residue
Wash with 1 L water
Filter
Filtrate
Residue
Centrifuge at 480 x g for 2 hr
Supernatant
Residue (discard)
Heat in a 96°C water bath for 5 min.
Centrifuge at 480 x g for 10 min
Supernatant
Residue (discard)
Concentrate at 75°C under vacuum (reducing volume to 1/5 of the original)
Add absolute ethanol in an ice bath until ethanol concentration reached 75%
Cool to 5-10°C
Centrifuge at 480 x g for 5 min
Precipitate
Supernatant (discard)
Dissolve in 500 mL warm water with shaking
Add ethanol to a final concentration of 25%
Add 100 ml of 10% lead acetate in 25% ethanol
Centrifuge at 480 x g for 10 min
Supernatant
Precipitate (discard)
Add ethanol to 75% concentration in ice bath
Centrifuge at 5°C, 480 x g for 5 min
Precipitate
Supernatant (discard)
Dissolve in 100 mL water
Centrifuge at 4°C, 20,200 x g for 40 min
Supernatant
Precipitate (discard)
Add 10% Na2CO3 until pH reached 9.5
Centrifuge for 10 min
Supernatant
Precipitate (discard)
Centrifuge at 4°C, 20,200 x g for 40 min
Supernatant
Precipitate (discard)
Acidify with 20% acetic acid to pH 6.5
Cool to 5°C
Mix with 5 volumes of cold absolute ethanol
Centrifuge at 4°C, 480 x g for 10 min
Precipitate
Supernatant (discard)
Wash with cold absolute ethanol
Dry under vacuum
CB-1A (light brown powder)

Figure 1. Steps involved in separation and purification of CB-1A.

Approximately 10-20 mg CB-1A was injected subcutaneously into each of three rabbits (body weight, 4-5 kg) once a week. After three to six weeks, blood serum was separated from the blood, and tested for antibody formation using an immunodiffusion technique against the purified CB-1A. The antibody formed was then separated from the whole blood serum by centrifuging at 480 x g for 10 min and stored frozen for use in subsequent experiments. Serum samples which had been frozen for more than a year were found to be immunologically as active as the freshly prepared sera.

V.2.2. *Qualitative and Quantitative Determination of CB-1A Allergenicity*

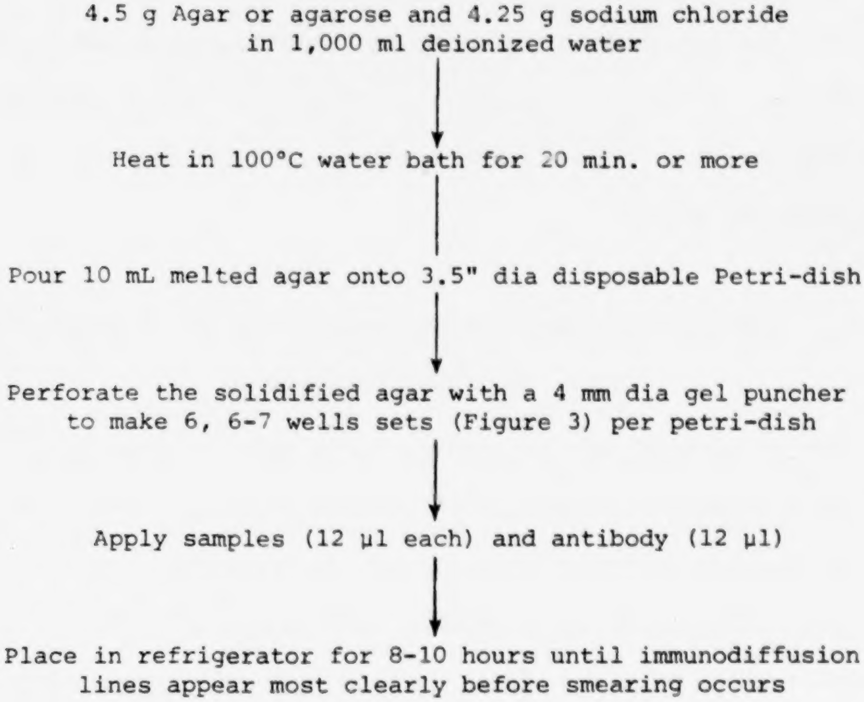
V.2.2.1. *Qualitative Determination*

Allergenic effects of castor seed products were determined quantitatively using the immunodiffusion technique of Ouchterlony (1968) with several modifications as described in Figures 2 and 3. Water extracts of castor seed products which contain crude CB-1A antigen were prepared routinely according to the procedure shown in Figure 4. For the qualitative analysis of allergen, the antigen extract was directly applied into peripheral wells against antibody in the center well (Figure 3).

The formation of one or more precipitin lines between center well (antibody) and the peripheral wells (antigen) was interpreted as the presence of allergenic antigen while the absence of precipitin lines was interpreted as the absence of the antigen, CB-1A. The minimum level of antigen needed to form detectable precipitin lines by naked eyes is not known at this time.

V.2.2.2. *Quantitative Determination*

For the quantitative estimation of the allergen, a dilution technique was applied to the double immunodiffusion method. The original or known amount of CB-1A solution was normally diluted to 1/2, 1/5, 1/10, 1/50, and 1/100 ratios.



4.5 g Agar or agarose and 4.25 g sodium chloride
in 1,000 ml deionized water

Heat in 100°C water bath for 20 min. or more

Pour 10 mL melted agar onto 3.5" dia disposable Petri-dish

Perforate the solidified agar with a 4 mm dia gel puncher
to make 6, 6-7 wells sets (Figure 3) per petri-dish

Apply samples (12 µl each) and antibody (12 µl)

Place in refrigerator for 8-10 hours until immunodiffusion
lines appear most clearly before smearing occurs

Figure 2. Procedures used for double immunodiffusion method.

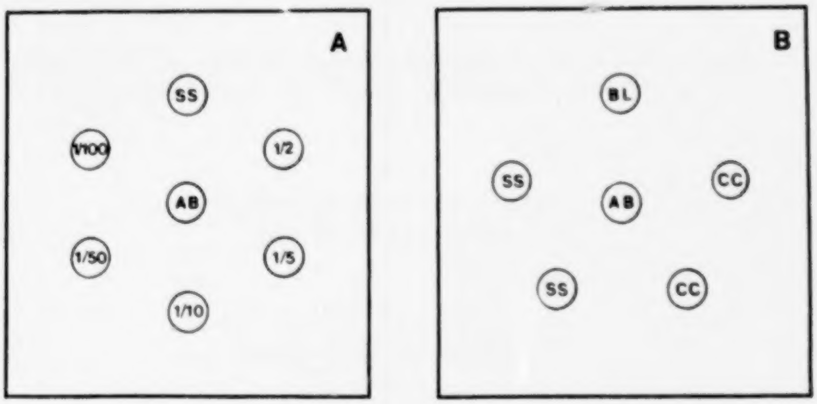
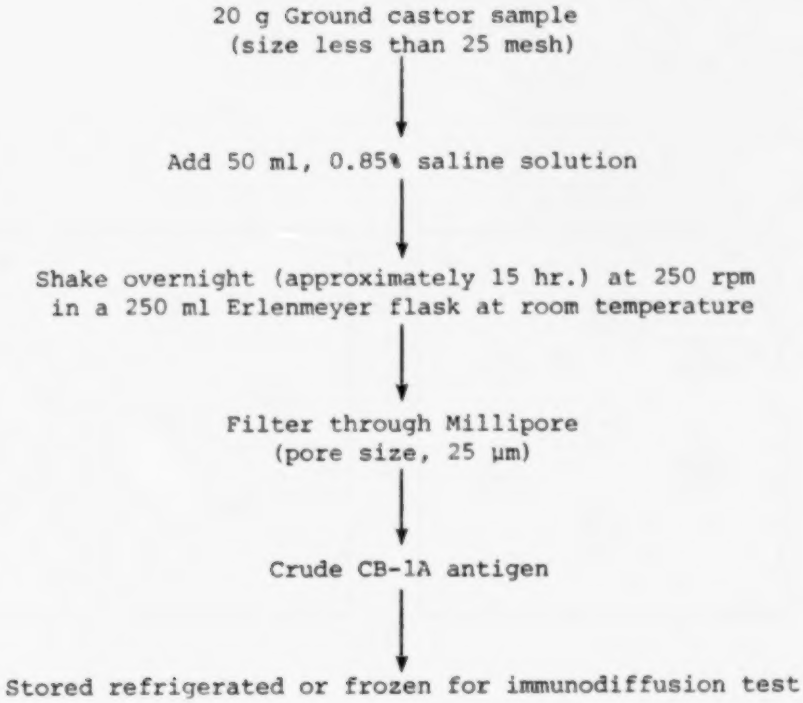

A
SS
1/100
1/2
AB
1/50
1/5
1/10
B
BL
SS
CC
AB
SS
CC

Figure 3. Sample illustration of wells perforated for double immunodiffusion (B) and dilution technique with double immunodiffusion (A). SS: sample, AB: antibody, BL:blank, CC: control, 1/2 - 1/100: dilution factor.



20 g Ground castor sample
(size less than 25 mesh)

Add 50 ml, 0.85% saline solution

Shake overnight (approximately 15 hr.) at 250 rpm
in a 250 ml Erlenmeyer flask at room temperature

Filter through Millipore
(pore size, 25 μm)

Crude CB-1A antigen

Stored refrigerated or frozen for immunodiffusion test

Figure 4. Preparation of CB-1A antigen for use in double immunodiffusion test.

The peripheral wells were then filled with these diluted CB-1A solutions along with the unknown sample as demonstrated previously in Figure 4. After development of the precipitin lines between the antibody (center well) and the surrounding antigenes (peripheral wells), the dilution which showed the most closely matching precipitin line intensity with that of the unknown sample was used to estimate the relative amount of antigen present in the unknown sample.

V.2.3. *Ricin Toxicity Testing Method*

V.2.3.1. *Hemagglutinin Test*

Toxicity of ricin and other toxic substances was determined by the red blood cell agglutination test. The procedure of Gardner et al. (1960) was slightly modified to prepare the red blood cell corpuscle and sample extracts to carry out the hemagglutinin test. The steps involved in the preparation of red blood corpuscle are shown in Figure 5.

For ricin agglutination test, ten test tubes were placed in a rack and 0.9 mL of saline solution was pipetted into the first tube and 0.5 mL each in subsequent tubes. To the first tube was added 0.1 mL meal extract, and the content mixed using a Vortex mixer. One-half (0.5 mL) of this mixture was transferred to the second tube and the content mixed. The same procedure was repeated until all ten tubes were prepared. Then, 0.5 mL of red blood corpuscle solution was added to each tube and mixed vigorously. The dilutions of meal extracts in the tubes were 1:10, 1:20, 1:40 and so on. The contents of the tubes were centrifuged at 500 x g for 2 min, and the appearance and characteristics of the red blood agglutination were observed after shaking the tube gently. The following ratings were then made:

+4: Complete agglutination with no dispersion by gentle shaking.

+3: Complete agglutination with some breaking up by gentle shaking.

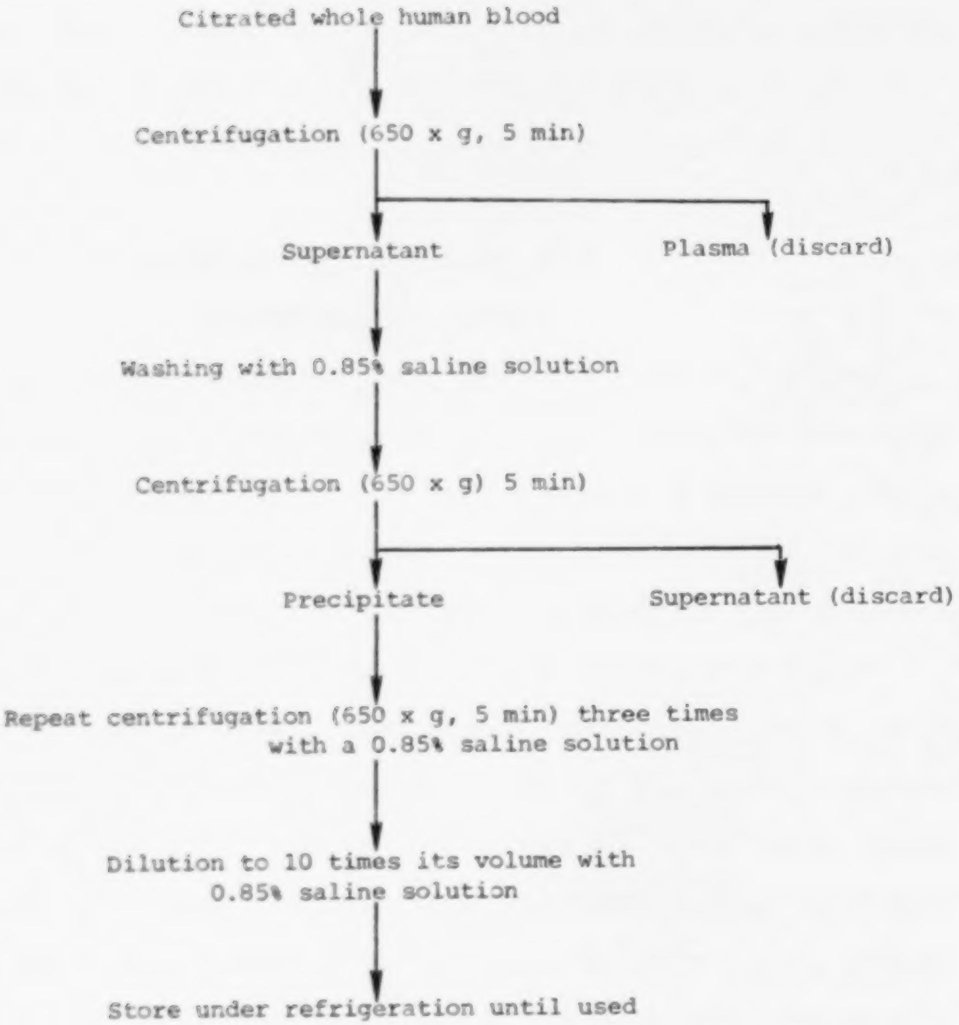

Citrated whole human blood
Centrifugation (650 x g, 5 min)
Supernatant
Plasma (discard)
Washing with 0.85% saline solution
Centrifugation (650 x g) 5 min)
Precipitate
Supernatant (discard)
Repeat centrifugation (650 x g, 5 min) three times with a 0.85% saline solution
Dilution to 10 times its volume with 0.85% saline solution
Store under refrigeration until used

Figure 5. Preparation of red blood cell corpuscle for use in ricin agglutination test.

+2: Agglutination, but complete breaking up of blood in the agglutinated particles.

+1: Agglutination, but easily dispersed to visible agglutinated particles.

The highest dilution showing +1 rating was defined as the "titre". The value of titre represents the amount of ricin present in the sample extract. The procedures used for ricin agglutination test are summarized in Figure 6.

V.2.4. *Evaluation of Chemical Treatment and Extrusion*

V.2.4.1. *Detoxification and Deallergenation of Commercially Defatted Castor Meal*

Based on the results obtained during the Phase II studies, the effectiveness of several oxidizing and/or alkaline chemicals in castor meal detoxification and deallergenation was retested using commercial extruders. Three types of extruders, Wenger X-20, X-25, and X-200, with rated throughputs of about 250, 1,000, and 6,750 kg castor meal per hour, respectively, were employed as continuous high temperature-short time reactors to accelerate the detoxification and deallergenation reactions of the chemicals. The general flow diagrams of the procedures are shown in Figures 7 and 8.

To confirm the findings of the phase II studies, a series of confirmatory test runs (Run II) were made with the commercially defatted castor meal obtained from West Germany using the X-20 extruder at Texas A&M University. The detailed conditions used for the test are summarized in Figure 7.

The final scaled-up testings (Run III, Run IV, Run V) were made at the Extruder Testing Laboratory of Wenger Manufacturing Company in Sabetha, Kansas (Figure 8) using the same commercially defatted meal and the Wenger X-20, X-25, and X-200 extruders. First, a series of short, about 30 min, runs were made with each extruder to again confirm the preliminary findings of the procedure. These test runs were followed by a series of sustained runs to evaluate the feasibility of commercial-scale operations, to produce enough quantities of

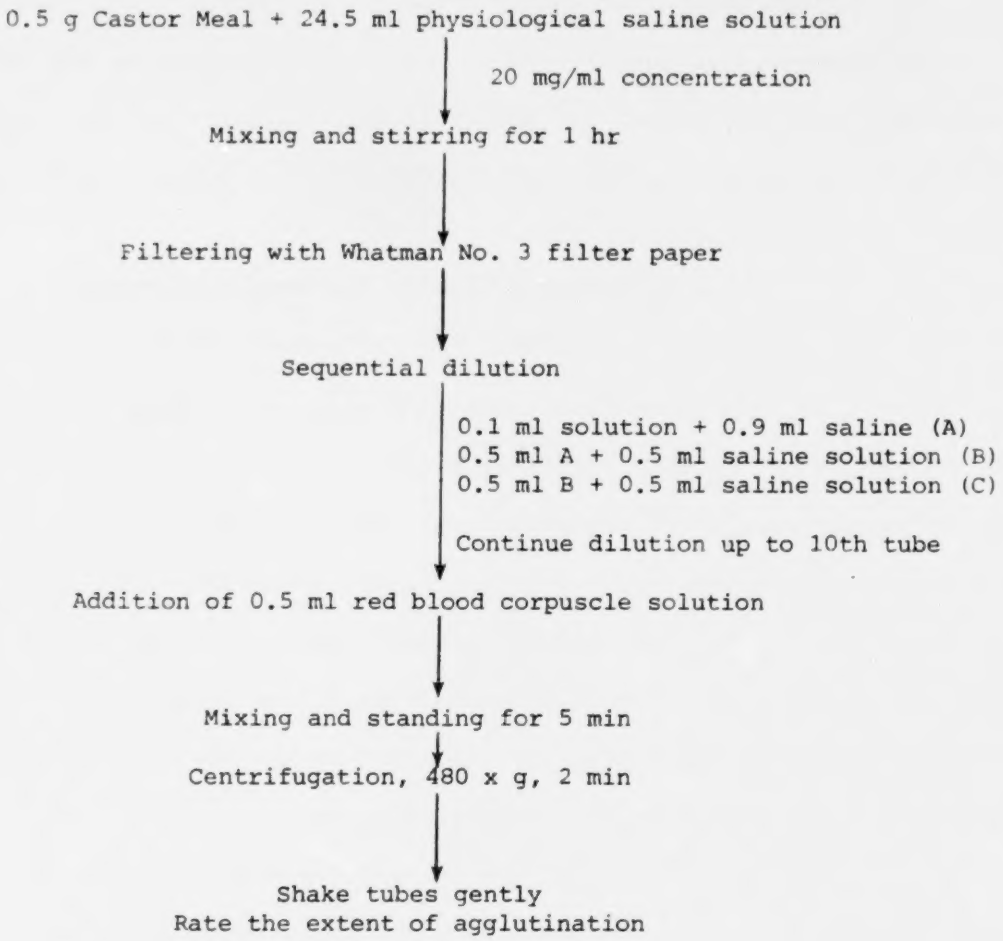

0.5 g Castor Meal + 24.5 ml physiological saline solution
20 mg/ml concentration
Mixing and stirring for 1 hr
Filtering with Whatman No. 3 filter paper
Sequential dilution
0.1 ml solution + 0.9 ml saline (A)
0.5 ml A + 0.5 ml saline solution (B)
0.5 ml B + 0.5 ml saline solution (C)
Continue dilution up to 10th tube
Addition of 0.5 ml red blood corpuscle solution
Mixing and standing for 5 min
Centrifugation, 480 x g, 2 min
Shake tubes gently
Rate the extent of agglutination

Figure 6. Procedures used for ricin agglutination test.

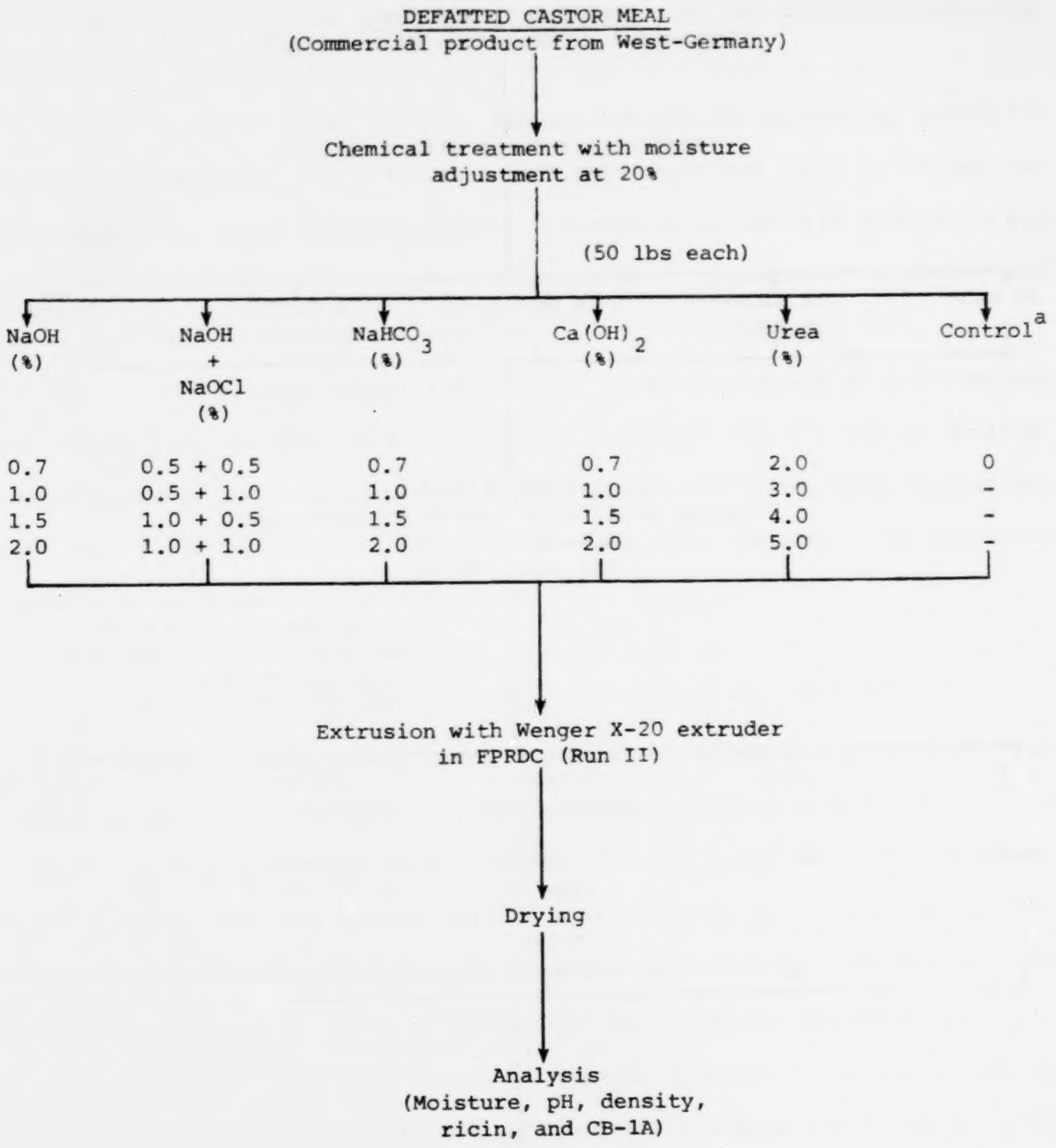

DEFATTED CASTOR MEAL
(Commercial product from West-Germany)
Chemical treatment with moisture adjustment at 20%
(50 lbs each)
NaOH (%)
NaOH + NaOCl (%)
NaHCO3 (%)
Ca(OH)2 (%)
Urea (%)
Control a
0.7
1.0
1.5
2.0
0.5 + 0.5
0.5 + 1.0
1.0 + 0.5
1.0 + 1.0
0.7
1.0
1.5
2.0
0.7
1.0
1.5
2.0
2.0
3.0
4.0
5.0
0
-
-
-
Extrusion with Wenger X-20 extruder in FPRDC (Run II)
Drying
Analysis
(Moisture, pH, density, ricin, and CB-1A)

Figure 7. Preliminary testing (Run II) for pilot scale extrusion of defatted castor meal. [a]Moisture adjustment to 20% (w/w) only.

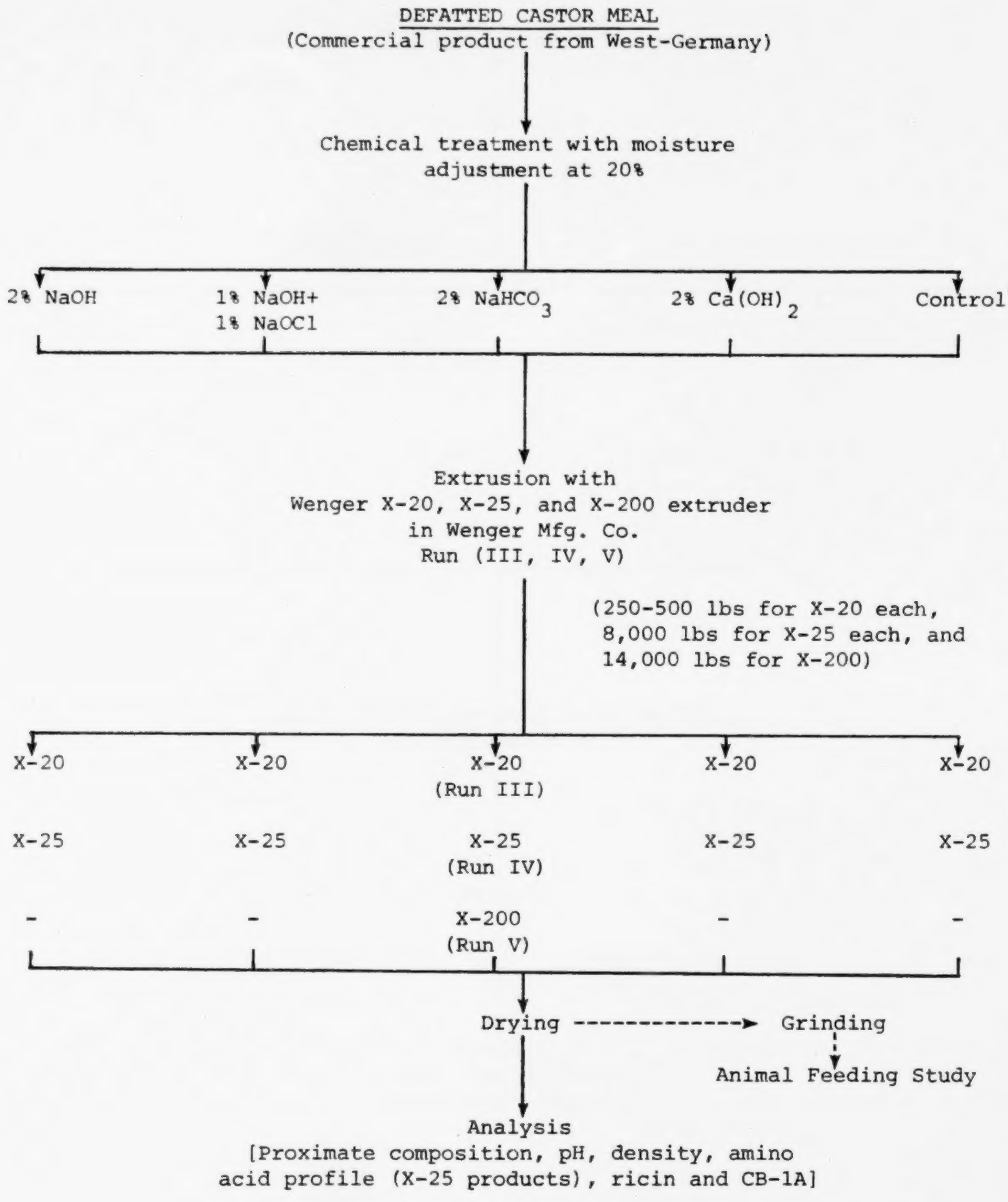

DEFATTED CASTOR MEAL
(Commercial product from West-Germany)
Chemical treatment with moisture adjustment at 20%
2% NaOH
1% NaOH+ 1% NaOCl
2% NaHCO3
2% Ca(OH)2
Control[a]
Extrusion with Wenger X-20, X-25, and X-200 extruder in Wenger Mfg. Co. Run (III, IV, V)
(250-500 lbs for X-20 each, 8,000 lbs for X-25 each, and 14,000 lbs for X-200)
X-20
X-20
X-20 (Run III)
X-20
X-20
X-25
X-25
X-25 (Run IV)
X-25
X-25
-
-
X-200 (Run V)
-
-
Drying
Grinding
Animal Feeding Study
Analysis
[Proximate composition, pH, density, amino acid profile (X-25 products), ricin and CB-1A]

Figure 8. Production testings (Runs III, IV, and V) for commercial scale extrusion of defatted castor meal. [a]Moisture adjustment to 20% (w/w) only.

samples for animal feeding tests, and to gather operational information needed to make preliminary designs for production plants with two different throughput capacities.

Throughout the extruder testing, various operational parameters needed for effective operation of the extruders, such as number and types of screws and heads, feed rate, steam and water supply in mixing zone, speed of main drive and mixing cylinder, temperature control of each zone, operational stability and production rate were checked and recorded.

The extrudates were either air-dried at room temperature in a forced-air hood (Texas A&M) or machine-dried (Wenger) at 107°C for 20 min to a final average moisture contents of about 5-10%. Dried extrudates were tested for ricin and CB-1A activities, pH of water extracts, density, and proximate composition, as needed.

V.2.4.2. *Detoxification and Deallergenation of Prepressed Castor Cake*

Two batches of prepressed castor cakes with different oil contents were produced using a Simon-Rosedowns' expeller. Castorseeds were preconditioned at 120°C for 15 min in a Bauer 22 inch cooker. Enough water (water-to-seed ratio of about (1:50, w/w) was sprayed on the seed prior to preconditioning so that the final moisture content of the preconditioned seed was approximately 4%. The cooker was pre-heated by steam to about 150°C before adding the seeds.

The prepressed cakes were ground into powder with Kolloplex and Contraplex pin mills and then treated with sodium hydroxide + sodium hypochlorite (1+1%, w/w) based on a final moisture content of 20%. The extrusion test was carried out using the Wenger X-20 extruder at Texas A&M University following the identical procedure used for the commercially defatted castor meal (Figure 7).

The extent of detoxification and deallergenation was then determined using the same hemagglutinin and immunodiffusion methods as previously described. The residual oil content of the extrudate was determined by the acid hydrolysis oil determination method (AOAC, 1980).

V.2.5. *Characterization of Extruded Products*

V.2.5.1. *Proximate Analysis*

All samples were analyzed using the Official Methods of AOAC (1980) and AOCS (1969). Moisture and solid contents were determined by the AOAC method 14.004; ash, AOAC method 14.006; crude nitrogen and protein, AOCS method Aa 5-38; crude fat, AOCS method Aa 4-38; and crude fiber, AOCS method Ba 6-61.

V.2.5.2. *Amino Acid Analysis*

Samples (200 mg) were hydrolyzed in 100 mL 6N HCl by refluxing for 24 hr. The hydrolyzates were dried using a rotary evaporator at 40°C, then dissolved in 2 mL sodium citrate buffer (pH 2.2). Samples (1 mg/mL) were taken for amino acid analysis using a Beckman 120C amino acid analyzer. The barium hydroxide technique of Moore et al. (1958) was used to prepare hydrolysate for tryptophan assays. Cystine was analyzed after oxidation with performic acid as described by Schram et al. (1954). Tryptophan was determined by the $Ba(OH)_2$ hydrolysis method as described by Kohler and Palter (1967).

V.2.5.3. *Density Measurement*

Extruded particles were placed into a 500 mL graduated cylinder half filled with rapeseed. Then the cylinder was filled with rapeseed, tapped three times on the table, and more seeds were added to keep the 500 mL level. The cylinder was then emptied, and refilled only with rapeseed. The difference between the original volume (500 mL) and that of rapeseed alone was the volume occupied by the extruded samples. The density was finally calculated by dividing the weight by the volume as below.

Density (g/mL) = weight of sample (g)/volume of sample (mL).

V.2.5.4. *pH Measurement*

A 20-g sample of finely ground extrudate was dispered into 50 mL distilled water in a 200 mL capped-Erlenmeyer flask. The slurry was placed on a shaker for 30 min at 200 rpm and then the relative pH was determined for each product.

V.2.6. ***Biochemical Evaluation of CB-1A***

V.2.6.1. *Determination of Sulfhydryl (SH) and Disulfide (SS) Groups*

The assay procedure reported by Beveridge et al. (1974) was used to determine the contents of both SH and SS groups using the Ellman's reagent. The purified CB-1A (200 mg) was dissolved in 10 mL of 1% NaCl in Tris-Glycine buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0). For determination of groups, 2.9 mL of 0.5% SDS in Tris-Glycine buffer, 0.1 mL of the diluted CB-1A solution and 0.02 mL of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid in Tris-Glycine buffer, 4 mg/mL) were mixed for color development. For determination of disulfide groups, 0.2 mL of the diluted CB-1A solution, 1 mL of 10 M urea in Tris-Glycine buffer and 0.02 mL of 2-mercaptoethanol were mixed and allowed to stand for 1 hr at 25°C. After an additional 1 hr incubation with 10 mL of 12% TCA, the tubes were centrifuged at 5,000 x g for 10 min. The precipitate was twice re-suspended in 5 mL of 12% TCA and centrifuged to remove 2-mercaptoethanol. The precipitate was dissolved in 3 mL of 0.5% SDS in Tris-Glycine buffer, then 1 mL was diluted to 10 mL with the same SDS solution. Color was developed by adding 0.05 mL of Ellman' reagent. Absorbance was measured at 412 mm on a Beckman DU-6 spectrophotometer.

V.2.6.2. *Rocket Immunoelectrophoresis*

The method described by Axelsen and Svendsen (1973) was followed. The step-by-step procedures are summarized in Figure 9.

V.2.6.3. *Disc Gel Electrophoresis*

The procedure reported by Davis (1964) was followed for the disc gel electrophoresis. Briefly, the protein sample (0.04 mL of 1% solution in Tris buffer, pH 8.6) was loaded on the top of the sample gel. The electrophoresis was carried out using a running gel composed of 7.5% acrylamide at a current of 3 mA/gel for about 2 hr, until the tracking dye (bromophenol blue) reached 10 mm above the gel bottom.

V.2.6.4. *SDS Acrylamide Gel Electrophoresis*

Basically, the procedure reported by Weber and Osborn (1975) was used to carry out the SDS acrylamide gel electrophoresis. Briefly, the protein sample (0.04 mL of 0.1% solution in phosphate buffer, pH 7.1) was loaded on the top of the running gel (12% acrylamide). The electrophoresis was carried out at a current of 8 mA/gel. Normally, 8 hr was taken until the tracking dye (coomassie brilliant blue) reached about 10 mm above the gel bottom.

The molecular weight of the sample protein (CB-1A) was determined by comparing its electrophoretic mobility with those of marker proteins (Sigma MW-SDS-17 Kit) with known molecular weights ranging between 2,510 and 16,950 (from Sigma Chemical Company, St. Louis, MO). The relative mobility (Rf) was then calculated as below.

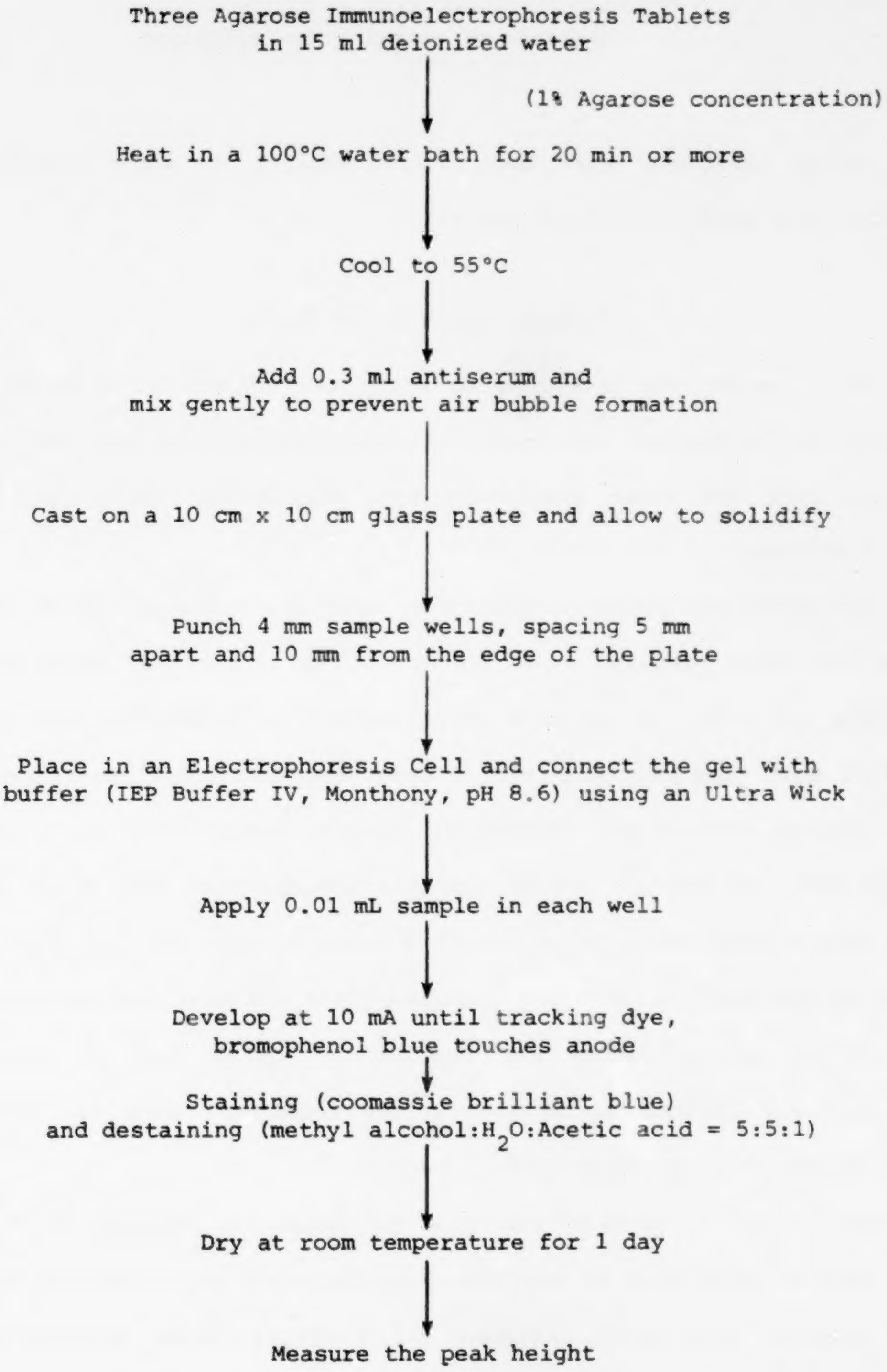

Three Agarose Immunoelectrophoresis Tablets in 15 ml deionized water
(1% Agarose concentration)
Heat in a 100°C water bath for 20 min or more
Cool to 55°C
Add 0.3 ml antiserum and mix gently to prevent air bubble formation
Cast on a 10 cm x 10 cm glass plate and allow to solidify
Punch 4 mm sample wells, spacing 5 mm apart and 10 mm from the edge of the plate
Place in an Electrophoresis Cell and connect the gel with buffer (IEP Buffer IV, Monthony, pH 8.6) using an Ultra Wick
Apply 0.01 mL sample in each well
Develop at 10 mA until tracking dye, bromophenol blue touches anode
Staining (coomassie brilliant blue) and destaining (methyl alcohol:H_2O:Acetic acid = 5:5:1)
Dry at room temperature for 1 day
Measure the peak height

Figure 9. Procedure used for rocket immunoelectrophoresis.

$$Rf = \frac{\text{distance of sample migration}}{\text{distance of tracking dye migration}}$$

The Rf values (abscissa) were then plotted against the known molecular weight (ordinate) on a semi-logarithmic paper.

V.2.6.5. *Model System Studies*

A model system was developed to study the effects of chemical and heat treatments on biochemical and immunological properties of purified CB-1A. The conditions used for these treatments were similar to those used in actual extrusion studies.

To determine the property changes by disc gel electrophoresis, a 1% CB-1A solution was first prepared by dissolving 0.1 g of purified CB-1A into 9.9 mL Tris buffer (pH 6.7). An aliquote of this solution (1 mL) was then transferred to a 10-mL test tube and heat-treated under the conditions summarized in Table 1. To prepare a heat and chemically treated sample, 25 µL of 10% sodium hydroxide solution and 10% sodium hypochlorite solution were first mixed with 9.85 mL Tris buffer (pH 6.7) to obtain a solution with 250 ppm each chemical, followed by the addition of 0.1 g purified CB-1A and heat treatment as shown in Table 1. The heated sample was immediately cooled down in ice-water and refrigerated for further analysis. Various conditions used for the heat and chemical treatments are summarized in Table 2.

Determination of protein subunits and molecular weights of the treated samples were accomplished by the SDS polyacrylamide gel electrophoresis. The treated samples were also analyzed for residual CB-1A activities by the immunodiffusion method and amino acid composition.

Table 1. Heat treatment conditions for the model system studies

	Disc gel electrophoresis (1% CB-1A)	SDS acrylamide gel electrophoresis (0.1% CB-1A)
Heat treatment only		
A (control)	No treatment	15 min, 60°C[a]
B	120 min, boiling water (100°C)	120 min, boiling water
C	10 min, autoclaving (120°C)	10 min, autoclaving (120°C)[b]
D	30 min, autoclaving (120°C)	
Heat & Chemical Treatment		
E (control)	No treatment	15 min, 60°C[a]
F	1/3 min, boiling water (100°C)	10 min, autoclaving (120°C)[b]
G	2/3 min, boiling water (100°C)	
H	1 min, boiling water (100°C)	

[a] Incubation.

[b] Heating after incubation.

Table 2. Treatment conditions used for the model system studies[a]

	Temp (°C)	Time (min)						
1.	50	5	7.5	10				
2.	60	5	7.5	10				
3.	70	0.7	1	1.5	2	2.5	3	3.5
4.	80	0.7	1	1.5	2			
5.	90	0.3	0.7	1				
6.	100	0.3	0.7	1				
7.	control	0						

[a] The general schemes for heat and chemical treatment were identical to the method previously described for the preparation of disc gel electrophoresis samples. The amount of residual CB-1A was estimated by both the dilution technique of the double immunodiffusion and the rocket immunoelectrophoresis.

V.2.7. ***Animal Feeding Tests***

Rat feeding test was carried out at the Hazleton Laboratories America, Inc. in Madison, Wisconsin to determine the Protein Efficiency Ratio (PER) of the treated castor meal. Chick (Experiments I, II and III) and swine feeding tests were carried out at the Animal Testing Center facilities of the Texas A&M University in College Station, Texas to determine the efficacy of the treated castor meals for the growth and performance of these animals. The histological examination of a selected number of chick and swine tissues was conducted at Texas Veterinary Medical Diagnostic Laboratory of Texas A&M University in College Station, Texas.

V.2.7.1. *Rat Feeding Tests*

The Official Method of AOAC (1980), Method 43,212, was followed using ANRC Reference Casein as the control for the study.

The test was conducted with 30 rats per treatment. Test rats were fed isonitrogenous diets containing 25% treated castor meal as the only source of protein for 28 days. Feed and water were furnished ad libitum. Feed consumption and weight gain were determined weekly, and mortality was determined daily.

V.2.7.2. *Chick Feeding Tests*

V.2.7.2.1. Battery Brooder Study (Experiment I)

An experiment was designed to test the effects of various castor bean meal products on growth, feed efficiency and mortality in battery brooder reared broilers. A total of 640 male Indian River broiler chicks were randomly assigned to the treatments shown in Table 3 (Chick test, Exp. I). There were 4 replications of 10 birds each for a total of 64 pens.

Table 3. Formulation of the diets for the chick and swine feeding tests

Diet #	Castor meal, %				Treatment
	Chick test			Swine test	
	Exp. 1	Exp. II	Exp. IV		
0	0, 0 & 0	0	0	0	Control
1	4, 8 & 12	10	5 or 10	10	Untreated
2	4, 8 & 12	10	5 or 10	10	2% NaOH
3	4, 8 & 12	10	5 or 10	10	2% $NaHCO_3$
4	4, 8 & 12	10	5 or 10	10	2% $Ca(OH)_2$
5	4, 8 & 12	10	5 or 10	10	1% NaOH + 1% NaOCl

Each castor meal product was fed in combination with corn and soybean meal so that all diets contained 23% crude protein and 3,180 Kcal/kg of poultry metabolizable energy. Mortality records were kept daily and both feed consumption and growth were recorded at weekly intervals for period of 34 weeks. Histological examination was made on selected tissues from the control group and birds fed 12% castor meal. Data were anlayzed using the GLM ANOVA procedure of the statistical analysis system.

V.2.7.2.2. Floor Pen Study (Experiment II)

A total of 1,500 one day old male Indian River broiler chicks were equally divided among six 2.44 m x 3.05 m (8 ft x 10 ft) broiler floor pens so that each pen contained 250 birds. The birds were brooded together for 10 days before establishing the five replications because of the cold weather during the initial experimental period. Each pen of broilers were fed one of the broiler starter diets shown in Table 3 (Chick test, Exp. II).

At the end of the brooding period, the birds were divided among 30 floor pens, 48 birds per pen. This allowed each diet to be replicated 5 times. Some birds were culled so that each pen contained an equal number at day 10.

A two diet broiler feeding protocol consisting of a starter/grower and a separate finisher was utilized for this study. Both the control diets and the castor bean diets were formulated to be isocaloric and isonitrogenous. The castor meal was assigned a protein value of 39.6% and a poultry metabolizable energy value of 1,540 Kcal/kg for the purpose of diet formulation. After each pen had consumed a total of 68 kg (150 lbs) of the starter/grower diet (approximately 26 days) the finisher diets were fed for the remainder of the experiment.

Each pen of broilers was weighed at 29, 43, and 50 days of age. Total feed consumption to date was also determined at these times. Mortality was noted daily throughout the experiment. At day 50, various tissues were collected from birds within each treatment for histological analysis.

Analysis of variance was applied to weight gain and feed efficiency for each weight period. Feed efficiency was corrected for mortality using standard consumption tables. Duncan's Multiple Range Test was used to separate means when statistical significance was indicated.

V.2.7.2.3. Laying Hen Study (Experiment III)

In this experiment, two hundred and twenty 26 week-old Shaver White Leghorn hens were distributed; 2 birds per cage in commercial type single-deck laying cages. The birds were separated and weighed into eleven groups and each group was subdivided into two replicates of 10 birds.

Corn-soybean meal diets were formulated to contain 5 to 10% of either untreated or treated meal as shown in Table 3 (Chick test, Exp. III).

The experimental diet in this study were isocaloric (2,748 M.E. Kcal/kg) and isonitrogenous (17.20% crude protein) and met NRC nutrient requirements.

Parameters measured weekly included rate of hen day, egg production, feed consumed, egg weight, yolk weight, yolk height, albumin height, albumin length, albumin width, yolk length, yolk width, egg color, shell thickness, and weight during the study.

Both feed and water were supplied ad libitum throughout the experimental period (6 weeks). Egg production and mortality were recorded daily. Five birds from each replicate were weighted biweekly and feed consumption was calculated at the end of the study.

Measurement were made of rate of hen day egg production, feed consumed, egg weight, yolk weight, egg color, shell thickness, and weight change during the study.

Analysis of variance was applied to each of the measured parameters for each weekly period. Duncan's Multiple Range Test was used to separate means when statistical significance was indicated.

V.2.7.3. *Swine Feeding Tests*

The five castor meals used for the test were designated as shown in Table 3 (Swine test). The corn and soybean meal used in the trial were obtained from commercial sources, and the same batches of soybean meal and corn were used throughout the trial.

The analyzed protein and lysine contents of the castor meals and soybean meal are given in Table 4. The analyzed lysine values were used in formulating the experiment diets (Table 3). The experimental diets were based on five castor meals and soybean meal. Ten percent castor meal was substituted for soybean meal in both the grower and finisher diets. The level of castor meal provided about 50% of the supplemental protein in the grower diets and about 70% of the supplemental protein in the finisher diets. Grower diets were formulated to 0.75% lysine and finisher diets were formulated to 0.60% lysine. Lysine-HCl was added to all castor meal diets to obtain the desired lysine levels.

Sixty crossbred pigs, initially averaging about 23 kg, were allotted to six outcome groups on the basis of weight, sex, and litter to provide five pens of two pigs each per outcome group. The outcome groups were then randomly assigned to the dietary treatments.

Pigs received feed and water ad libitum, and were weighed periodically throughout the experiment. The grower diets were fed during the first 47 days of the experiment and the finisher diets were fed thereafter. The two heaviest pigs (from one pen) on each diet were slaughtered after 88 days on test, the remaining pigs were slaughtered after 100 days on test.

Table 4. Analyzed protein and lysine contents of castor meals and soybean meal used in the swine growth trial[a]

	Castor bean meal					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Soybean meal
Protein, %	37.31	37.44	37.94	40.63	36.75	43.92
Lysine, %	1.52	1.13	1.21	1.12	1.00	2.89

[a] Values are on an as fed basis.

Pigs were slaughtered at the Texas A&M Meats Laboratory following standard slaughter and dressing procedure. As soon as the pigs were eviscerated, the following samples were taken and fixed in 10% buffered formalin: esophagus, stomach, duodenum, jejunum, ileum, caecum, spiral colon, descending colon, pancreas, liver, spleen, heart, lungs, kidney, urinary bladder and aesenteric lymph node. The same person sampled all viscera and care was taken to ensure that samples were taken from the same locations in the organs. The fixed samples were embedded in paraffin, sectioned and then stained with hematoxylin and eosin. The tissues were examined microscopically by Dr. William Schwartz, pathologist in the Texas A&M Veterinary Medical Diagnostic Laboratory in College Station, Texas.

V.2.8. *Preliminary Design of Production Plants*

The Wenger Manufacturing Company in Sabetha, Kansas was asked to develop equipment specifications for two castor meal treatment plants of 25 and 50 metric tons per 24-hr day capacities based on the information obtained during the pilot plant trials.

VI. RESULTS AND DISCUSSION

Laboratory studies, pilot size testings, and production scale operations for detoxification and deallergenation of castor meal were conducted to assess the technical feasibility of using selected chemicals and extrusion technology for such purposes. Additional objectives were to evaluate quantification methods for toxins and allergens, to study the biochemical and immunological changes in the major castorseed allergen during detoxification and deallergenation procedures, and to evaluate the acceptability and safety of the treated castor meals through limited animal feeding studies. Finally, based on the results obtained thus far, equipment layouts as well as preliminary designs of production plants with two different capacities were also included as the other objectives. The following characteristics were considered in evaluating the efficacy of the detoxification and deallergenation procedures.

a) Presence/absence of castorseed allergen, CB-1A, as indicated by the shape and thickness of precipitin line in double immunodiffusion method.

b) Presence/absence of major castorseed toxin, ricin, as indicated by *in vitro* blood agglutination method.

c) Physical and chemical properties of the products including pH, density, proximate composition, amino acid profile, reactive groups, and electrophoretic properties of the protein.

d) Overall acceptability and safety of products by the test animals.

VI.1. Proximate Composition of Raw Materials

Since commercial preparation of castor meals usually involves hulls which consist of 28% of the whole castorseed, the commercially defatted meal purchased from West Germany exhibited a high fiber content, 30.2%, as compared to (3.0%

fiber in defatted soy flour (Wolf, 1977). This high fiber content restricted the use of the detoxified and deallergenized castor meal in the feeds for test animals. Dehulling prior to oil extraction would be helpful to reduce the hull content of the meal, but it will be difficult to mechanically press the oil from dehulled seeds.

The protein content of the defatted castor meal, 37.3%, was relatively high although not quite as high as a commercially defatted soy flour, about 53% protein.

VI.2. Evaluation of CB-1A Allergenicity Testing

The purified CB-1A, in the form of a light brown powder, showed the typical three intense and two week bands in the disc gel electrophoresis. The rats intraperitoneally injected with 10-15 mg of the purified CB-1A showed acute abnormality. Approximately 10 mL of CB-1A anti-serum (antibody) was obtained per rabbit by subcutaneous injection of CB-1A along with an antibody-stimulator once a week for 3 to 6 weeks.

The precipitin reaction between antibody and antigen is a very sensitive in vitro immunochemical method to determine the reactivity of protein-like substances. Although this method is time-consuming, requiring preparation of pure CB-1A and its anti-serum (antibody), it is specific and reproducible once the technique has been mastered. However, interpretation of the resulting double immunodiffusion patterns for quantitation of the allergenecity reduction is still somewhat difficult. At present, the immunodiffusion method is the only reliable analytical tool available for determination of CB-1A.

VI.3. Evaluation of Ricin Toxicity Testing

The in vitro hemagglutinin reaction method which takes advantage of the ability of toxins to coagulate the red blood cell corpuscle has been proven effective in estimating the ricin toxicity. The Biuret method which is based on the protein solubility change caused by protein denaturation can also be used to estimate the ricin toxicity.

VI.4. Effects of Chemical Treatment and Extrusion on Residual CB-1A and Ricin

As discussed in the Final Report of Phase II studies, when castor seeds were processed through pre-conditioning, pre-pressing, solvent extraction and desolventization, ricin was destroyed completely and the hemagglutinin reaction was no longer detectable in the meal. Also, the CB-1A content of the meal has, in some instances, been reduced by as much as 90%, but the residual CB-1A remaining in the meal was still sufficiently large to cause a strong positive precipitin reaction. It was also reported that selected number of oxidizing and/or alkaline chemicals were very effective to further reduce the residual CB-1A content, when used with an extruder as a high-temperature short-time reactor to accelerate the chemical reaction.

VI.4.1. *Treatment of Commercially Defatted Castor Meal*

A series of confirmatory test runs (Run II), covering a wider range of chemicals and concentrations than that used in Phase II Studies were conducted at Texas A&M to verify the optimum process conditions found in Phase II studies using the commercially defatted castor meal.

These confirmatory runs were followed by a series of production runs (Run III, Run IV, and Run V) at the Wenger Manufacturing Company in Sabetha, Kansas. The extruders used for this study were Wenger X-20 (Run III), X-25 (Run IV), and X-200 (Run V) with throughputs of about 250, 1,000 and 6,750 kg meal per hour, respectively. The extruder configuration and operational conditions used for extrusion and chemical treatment of Run II, Run III, Run IV, and Run V are shown in Tables 5, 6, and 7, respectively.

Table 5. Extruder configuration used for detoxification and deallergenation of castor meal

	RUN II	RUN III	RUN IV	RUN V[a]
TYPE EXTRUDER	X-20	X-20	X-25	X-200
TYPE BIN	Live	Live	C.B.	C.B.
TYPE FEEDER			Long pitch	6"
TYPE MIXING CYL.	Double	Same as	Single	Double
EXTRUDER SHAFT	68638-5	Run II	7-head	7-head
INLET SCREW	68327-1		28638-1	31305-1
INLET HEAD	68714-1		28905-13	31301-1
1st STEAMLOCK	68364-1		28364-1	31318-1
2nd SCREW	68327-1		28326-9	31321-9
2nd HEAD	68372-1		28906-1	31319-1
2nd STEAMLOCK	68364-1		38364-1	31318-1
3rd SCREW	68327-1		28326-9	31321-9
3rd HEAD	68372-1		28906-13	31319-3
3rd STEAMLOCK	68364-1		28364-1	31318-1
4th SCREW	68327-1		28326-9	31321-9
4th HEAD	68372-1		28372-9	31319-5
4th STEAMLOCK	68364-1		28364-1	31318-1
5th SCREW	68327-1		28329-9	31321-9
5th HEAD	68372-1		28372-29	31319-3
5th STEAMLOCK	68364-1		28364-1	31318-1
6th SCREW	68327-1		28326-1	31695-3
6th HEAD	68372-1		28372-1	31319-3
6th STEAMLOCK	68364-1			31311-1
7th SCREW	68326-1			31395-1
7th HEAD	68372-1		28372-1	31303-29
7th STEAMLOCK				
8th SCREW	68372-1			
8th HEAD	68696-1		28882-1	31374-1
8th STEAMLOCK				
SCREW CLEARANCE FROM END				
DIE SPACERS	68696-1		28882-1	31374-1
1st DIE #/DESCRIPTION				
CENTER DIE #/DESCRIPTION				
FINAL DIE #/DESCRIPTION	40 @1/4" dia.		28349-521	31328-69
INSERT NUMBER				
QUANTITY OF INSERTS			9 @7/32" dia.	15
DIE SET NUMBER	R-3-83			
TYPE KNIFE BLADES	19430-1		19512-3	19512-3
NUMBER KNIFE BLADES	6		6	16
KNIFE BLADE HOLDER NUMBER			19408-3	19520-1

INSERT DESCRIPTION: [a] Inserts had 5 holes @17/64" dia. x 1/4" thick.

COMMENTS:

Table 6. Extruder conditions used for detoxification and deallergenation of castor meal

Extruder Parameter	Run II (FPRDC)	Run III	Run IV	Run V
		(Wenger Manufacturing Co.)		
	X-20	X-20	X-25	X-200
Main drive				
Speed (rpm)	400	400	420	263
Current (amps)	50	22	56	250
Temp control				
Direct steam injection				
Mixing cylinder	yes	yes	yes	yes
Extruder head (Hd #)	yes (#3)	yes (#3)	yes (#3)	yes
Indirect steam heating				
Extruder head (Hd #)	3-7	4-6	5,6	1-3,6,7
Water cooling (Hd #)	2,8	1,2,7	1-4,7	4,5
Temp (°F)				
Mixing cylinder	165	165	145	120
5th Head	NA	280	228	NA
7th Head	260	330	NA	NA
8th Head	290	290	NA	NA
Feeder speed (rpm)	35	47	40	82
Water injection (rotometer set)				
Mixing cylinder	no	9.0	3.2	NA
Inlet head	no	7.1	135	NA
Dryer				
Temp (°F)	-	225	290	no
Retention time (min)	-	20	15.85	no

(Table 6 continued)

Extruder Parameter	Run II (FPRDC) X-20	Run III (Wenger Manufacturing Co.) X-20	Run IV X-25	Run V X-200
Moisture content of product (%)				
Pre-extruder	20.0	19.8	21.4	20.0
Off-extruder	24.3	35	31.5	21.6
Off-dryer	9.4	12	8.1	2.8
Production rate (lbs/hr, wet)	500	720	2,760	9,000

Table 7. Types and concentrations[a] of chemicals used in conjunction with extrusion for detoxification and deallergenation of defatted castor meal

Chemical	Concentration, %			
	Run II	Run III	Run IV	Run V
	(FPRDC)	(Wenger Manufacturing Co.)		
	X-20	X-20	X-25	X-200
NaOH	0.7	2.0	2.0	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
NaOH + NaOCl	0.5 + 0.5	1.0 + 1.0	1.0 + 1.0	-
	0.5 + 1.0	-	-	-
	1.0 + 0.5	-	-	-
	1.0 + 1.0	-	-	-
$NaHCO_3$	0.7	2.0	2.0	2.0
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
$Ca(OH)_2$	0.7	2.0	2.0	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
NaOCl	-	-	-	-
	-	-	-	-
H_2O_2	-	-	-	-
	-	-	-	-
Urea	2.0	-	-	-
	3.0	-	-	-
	4.0	-	-	-
	5.0	-	-	-
Control	Extrusion without chemical treatment			

[a] On a 20% moisture basis.

Table 8 shows the moisture content and the pH of the chemically treated and extruded products in Run II. As expected, the addition of alkaline chemicals caused the pH values of the product shift from neutral (raw material and control) to basic by 1 to 2 pH units.

Figure 10 shows the immunodiffusion patterns of the Run II extrudates. Treatment of castor meal with sodium bicarbonate and calcium hydroxide showed a gradual decrease in thickness of the antigen-antibody precipitin lines up to 1.5% chemical concentration. At 2% concentration, either a trace amount or no visual appearance of precipitin lines were observed for all samples. However, urea treatment was not effective at all, even at a 5% level.

The relative quantitative estimation of the residual CB-1A by dilution technique is shown in Figure 11. Extrusion of chemically treated samples of up to 1.0% level (A_1, A_2; B_1 B_2; C_1 C_2; and D_1, D_2) showed no more than 90% destruction (corresponding to 1/10 dilution) of the original amount of CB-1A. As the chemical concentration increases, a substantial amount of CB-1A (98-99%, corresponding to 1/50-1/00 dilution) was destroyed (A_3, A_4; B_3, B_4; C_3, C_4; and D_3, D_4). It seems that at least 1.5% chemical concentration is needed to reduce the CB-1A content to an acceptable level. However, a 2% chemical concentration is required for the maximum destruction of CB-1A, although there are slight variations among different types of chemicals. As mentioned earlier, urea was not effective at all regardless of its concentration (E_1-E_4).

Figures 12 and 13 show the immunodiffusion pattern and the quantitative estimation of residual CB-1A in the chemically treated (2% level) and extruded commercial castor meal (Run III and Run IV using X-20 and X-25 extruders at the Extrusion Testing Laboratory in Wenger Manufacturing Company, Sabetha, Kansas) . The results are identical to those previously obtained at Texas A&M. The X-20 which has less processing capacity showed better control of CB-1A deallergenation than the X-25. In this particular case, sodium hydroxide was

Table 8. Analytical data for extruded castormeal (Preliminary testings)

Sample[a]	Chemicals	Moisture (%)[b]	pH
	NaOH		
A_1	0.7%	25.6/14.5	7.5
A_2	1.0%	24.1/10.5	8.2
A_3	1.5%	25.8/14.1	8.9
A_4	2.0%	24.5/10.4	9.3
	NaOH/NaOCl		
B_1	0.5/0.5%	21.6/ 7.9	8.2
B_2	0.5/1.0%	25.4/11.5	7.8
B_3	1.0/0.5%	22.1/ 8.2	8.1
B_4	1.0/1.0%	23.2/ 8.7	8.0
	$NaHCO_3$		
C_1	0.7%	24.1/ 8.4	7.7
C_2	1.0%	23.8/ 8.2	7.7
C_3	1.5%	23.3/ 8.1	8.0
C_4	2.0%	21.5/ 9.0	8.3
	$Ca(OH)_2$		
D_1	0.7%	24.3/ 7.9	7.6
D_2	1.0%	24.6/12.8	7.8
D_3	1.5%	29.6/10.2	8.5
D_4	2.0%	22.8/ 7.7	7.5
	Urea		
E_1	2.0%	27.2/ 7.9	7.4
E_2	3.0%	25.1/ 6.8	7.3
E_3	4.0%	22.3/ 6.7	7.2
E_4	5.0%	23.0/ 6.6	7.2
	Control	25.7/11.3	7.2
	Raw material	11.6	7.2

[a] Designated in Figures 5 and 6.

[b] Before/after drying.

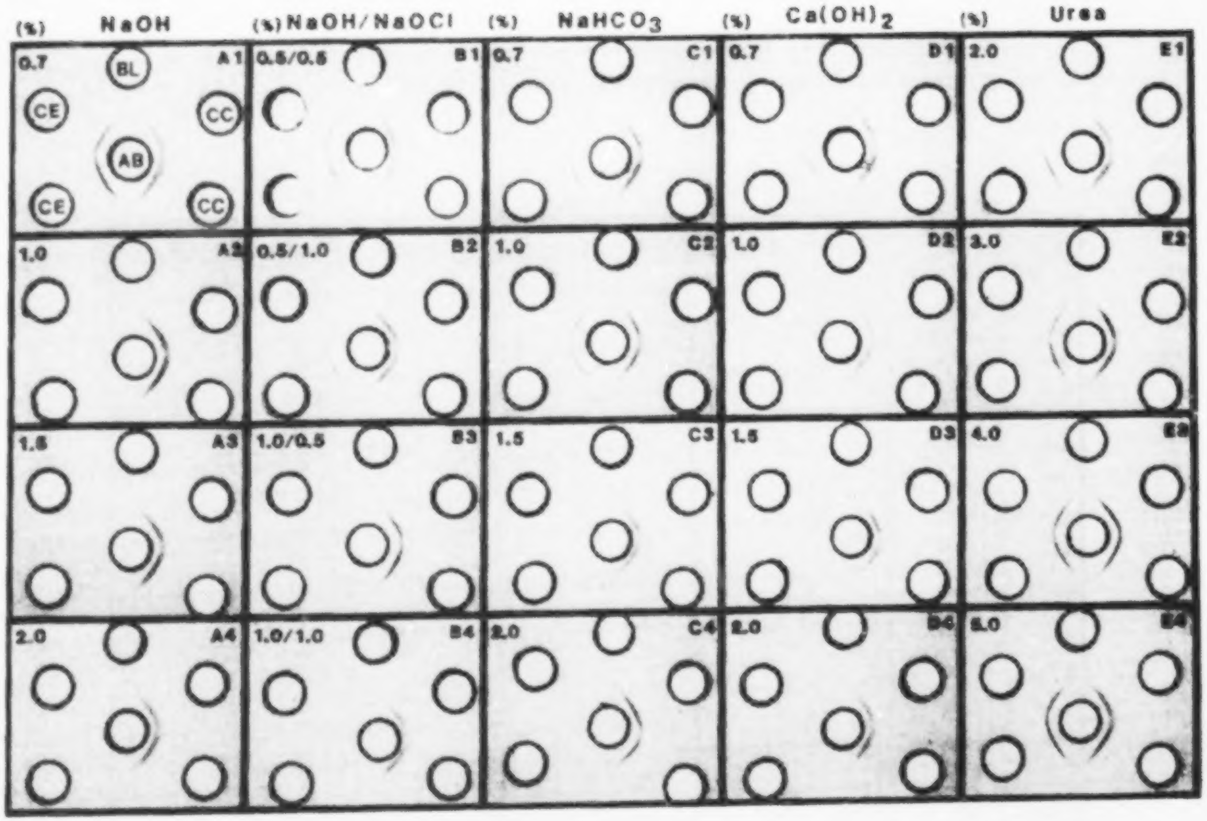

(%) NaOH
(%) NaOH/NaOCl
(%) NaHCO$_3$
(%) Ca(OH)$_2$
(%) Urea
0.7 A1 BL CE CC AB CE CC
0.5/0.5 B1
0.7 C1
0.7 D1
2.0 E1
1.0 A2
0.5/1.0 B2
1.0 C2
1.0 D2
3.0 E2
1.5 A3
1.0/0.5 B3
1.5 C3
1.5 D3
4.0 E3
2.0 A4
1.0/1.0 B4
2.0 C4
2.0 D4
5.0 E4

Figure 10. Immunodiffusion pattern of chemically treated and extruded (Run II) castor meals using different types and concentrations of chemicals. BL: blank; CC: control; CE: Sample; AB: antibody.

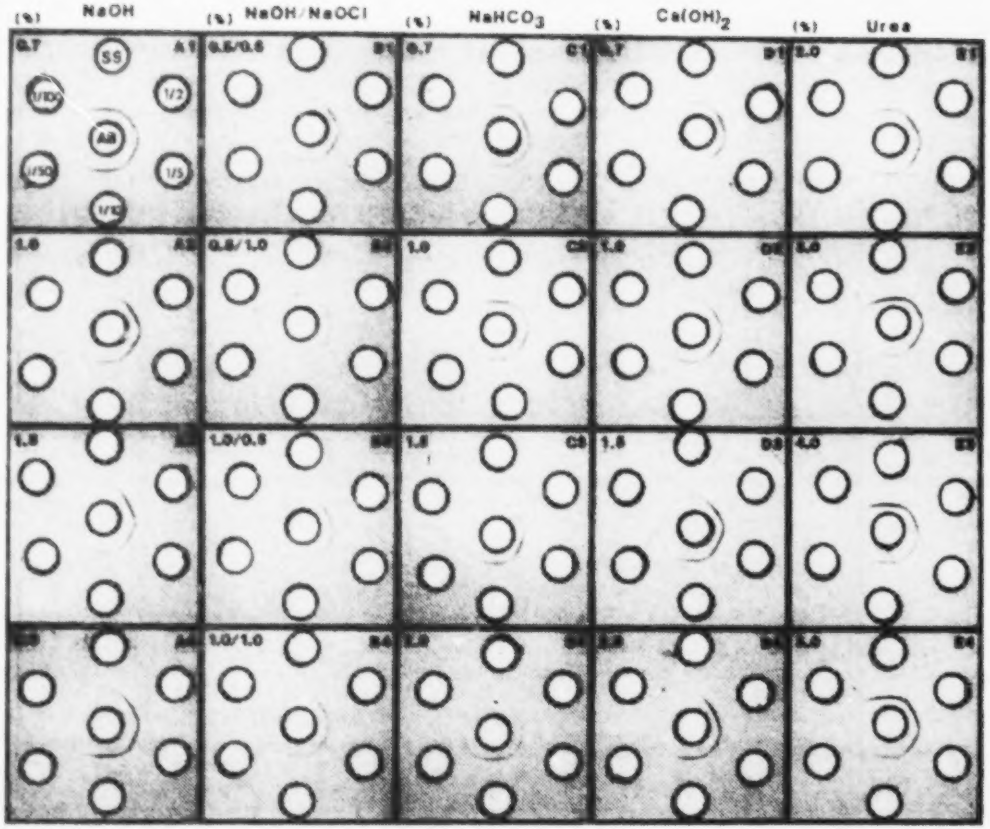

(%) NaOH
(%) NaOH/NaOCl
(%) NaHCO$_3$
(%) Ca(OH)$_2$
(%) Urea
0.7
A1
SS
1/2
1/5
1/10
1/50
1/100
AB
0.5/0.5
B1
0.7
C1
0.7
D1
2.0
E1
1.0
A2
0.5/1.0
1.0
1.0
1.5
1.0/0.5
1.5
C3
1.5
D3
4.0
1.0/1.0
B4

Figure 11. Dilution technique for quantitative estimation of CB-1A in chemically treated and extruded (Run II) castor meals. AB: antibody; SS: sample; 1/2 - 1/100: dilution factors.

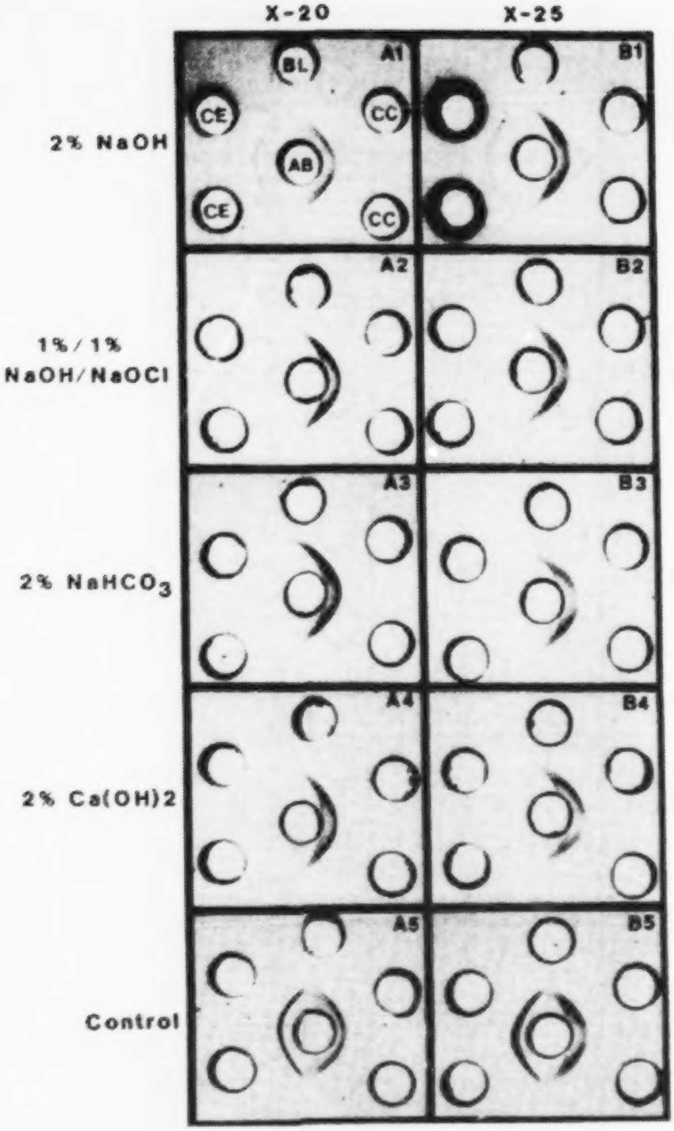

X-20
X-25
A1
B1
BL
CE
CC
AB
CE
CC
2% NaOH
A2
B2
1%/1%
NaOH/NaOCl
A3
B3
2% $NaHCO_3$
A4
B4
2% Ca(OH)2
A5
B5
Control

Figure 12. Effects of chemical treatment and extrusion (Run III) of the commercial castor meal on the precipitin reaction. BL: blank; CC; control; CE: sample.

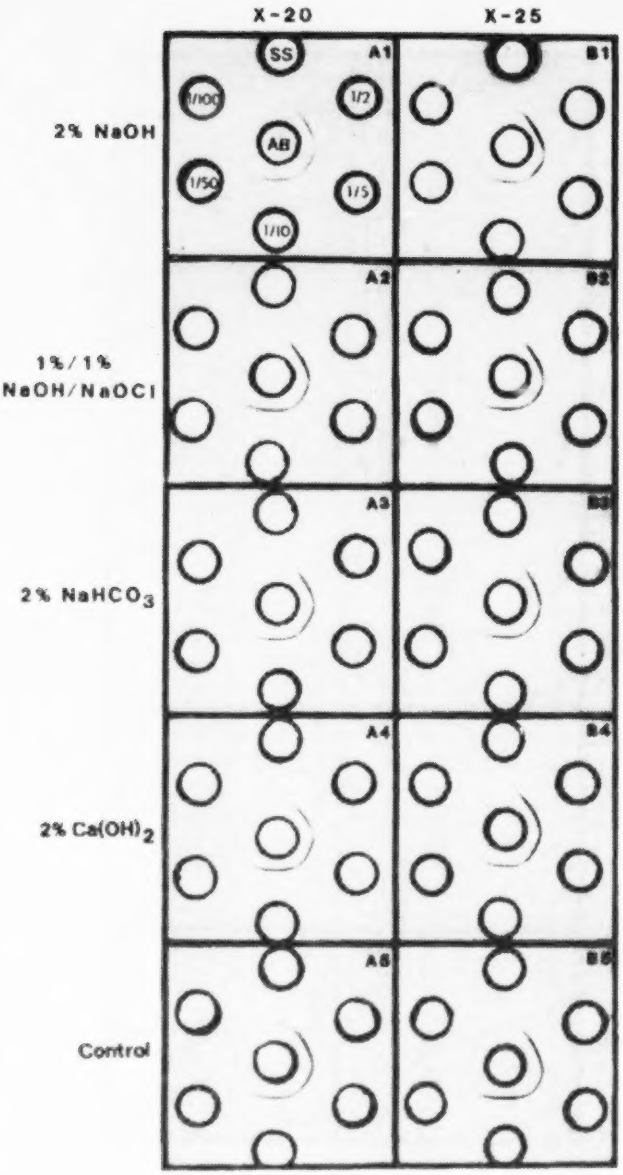

X-20
X-25
A1
SS
1/100
1/2
AB
1/50
1/5
1/10
B1
2% NaOH
A2
B2
1%/1% NaOH/NaOCl
A3
B3
2% $NaHCO_3$
A4
B4
2% $Ca(OH)_2$
A5
B5
Control

Figure 13. Estimation of the relative CB-1A concentrations remaining in the products after extrusion (Runs III and IV) of chemically treated commercial castor meal. SS: sample; AB: antibody; 1/2-1/100: dilution factors.

shown to be least effective of all chemicals. Two factors might have contributed to the poor performance of NaOH: one is the incomplete warming-up or thermal stabilization of the extruder during the initial stages of the operation, and the other being the inadequate mixing of NaOH with castor meal as the NaOH was directly injected to the mixing cylinder of the extruder rather than pre-mixing using a ribbon mixer.

Table 9 represents the tabulated data from Figures 10, 11, 12, and 13. It shows that extrusion without chemical treatment (control) could also reduce the residual CB-1A content by about 50% (X-20) to 80% (X-25). However, the combined action of chemical treatment (2%) and extrusion showed almost complete reduction (98-99%) of CB-1A originally present in the defatted meal.

Figure 14 shows the typical shape and size of the chemically treated and extruded products produced by using X-20 (A_1-A_5, Run III) and X-25 (B_1-B_5, Run IV) extruders. The added chemicals greatly affected the puffing characteristics of the extrudates, making the density of the product lighter, ranging from 0.43-0.75 g/cm^3 (Table 10). Another factor affecting the density of the product was the internal configuration of the extruder barrel and die size. In general, the X-25 extruder produced more puffed products than the X-20 extruder.

The maximum temperature attained in the X-25 extruder (127°C/260°F) was lower than that of X-20 (166°C/330°F), but the production rates were recorded as much as 900-1,250 kg (1,980-2,760 lbs) (cf. 327 kg/720 lbs for X-20) per hour on wet basis. This difference in the maximum temperature between X-20 and X-25 extruders does not seem to have any effect on the deallergenation reaction; however, the complete mixing of chemicals with castor meal prior to extrusion was quite essential for the effective chemical reaction.

Table 9. Effects of chemical treatment and extrusion on the reduction of CB-1A activity of castor meal

Chemical	Conc. (%)	Extruder Used	Estimated CB-1A Reduction, %	
NaOH	0.7	X-20	80	Run V
	1.0	X-20	90	Run V
	1.5	X-20	98	Run V
	2.0	X-20	98	Run V
	2.0	X-20	98	Run III
	2.0	X-25	98	Run IV
NaOH/NaOCl	0.5/0.5	X-20	90	Run V
	0.5/1.0	X-20	90	Run V
	1.0/0.5	X-20	98	Run V
	1.0/1.0	X-20	98	Run V
	1.0/1.0	X-20	99	Run III
	1.0/1.0	X-25	99	Run IV
$NaHCO_3$	0.7	X-20	50	Run V
	1.0	X-20	90	Run V
	1.5	X-20	98	Run V
	2.0	X-20	99	Run V
	2.0	X-20	98	Run III
	2.0	X-25	99	Run IV
	2.0	X-200	99	Run V
$Ca(OH)_2$	0.7	X-20	80	Run V
	1.0	X-20	98	Run V
	1.5	X-20	99	Run V
	2.0	X-20	98	Run V
	2.0	X-20	99	Run III
	2.0	X-25	99	Run IV
Urea	2.0	X-20	80	Run V
	3.0	X-20	80	Run V
	4.0	X-20	80	Run V
	5.0	X-20	80	Run V
Control	0.0	X-20	50	Run V
	0.0	X-20	90	Run IV
	0.0	X-25	80	Run IV

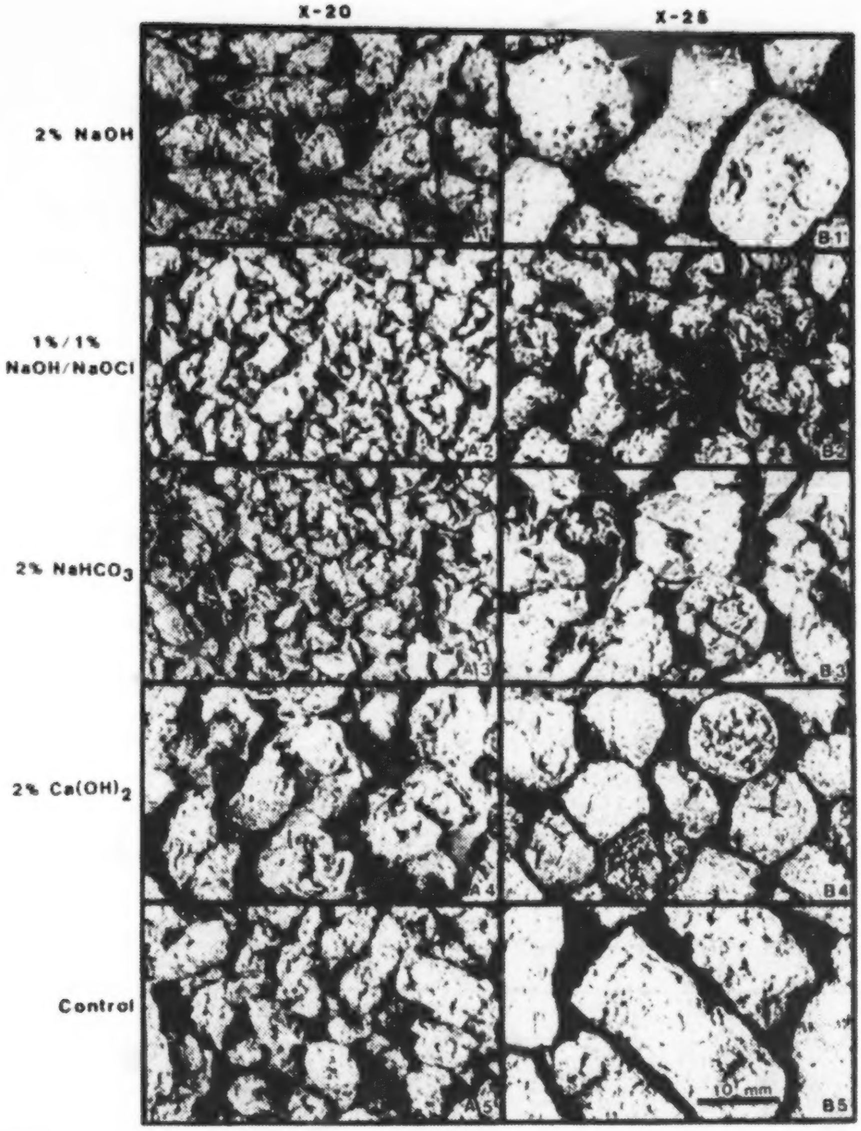

X-20
X-25
2% NaOH
1%/1%
NaOH/NaOCl
2% NaHCO3
2% Ca(OH)2
Control
A1
B1
A2
B2
A3
B3
A4
B4
A5
B5
10 mm

Figure 14. Shape and size of the chemically treated and extruded castor meal (Run III and Run IV).

Table 10. Density of chemically treated and extruded castor meal

	Treatment	Density (g/cm³)
X-20 (Run IV)	2% NaOH	0.62
X-20 (Run IV)	1%/1% NaOH/NaOCl	0.43
X-20 (Run IV)	2% $NaHCO_3$	0.42
X-20 (Run IV)	2% $Ca(OH)_2$	0.70
X-20 (Run IV)	Control, 20% moisture only	0.49
X-25 (Run IV)	2% NaOH	0.58
X-25 (Run IV)	1%/1% NaOH/NaOCl	0.58
X-25 (Run IV)	2% $NaHCO_3$	0.66
X-25 (Run IV)	2% $Ca(OH)_2$	0.75
X-25 (Run IV)	Control, 20% moisture only	0.63
X-200 (Run V)	2% $NaHCO_3$	0.75

VI.4.1.1. *Proximate Analysis*

The proximate analysis of the chemically-treated and extruded products from X-25 is shown in Table 11. All products, including the raw castor meal, have relatively high protein content (36.75-40.63%) and unusually high crude fiber content (26.32-31.28%). The amounts of ash in the chemically-treated and extruded products were slightly higher than the raw meal and control due to added chemicals.

VI.4.1.2. *Hemagglutinin Test*

No hemagglutinin reaction was present in the commercial castor meal imported from West Germany (Figure 15, picture C). It appears that ricin has already been destroyed completely during the preparation of this meal. Consequently, none of the chemically treated and extruded products made from the commercial castor meal exhibited any hemagglutinin reaction (Figure 15, pictures D-H). Accordingly, no more hemagglutinin tests were carried out when the commercial castor meal was processed.

VI.4.1.3. *Amino Acid Analysis*

Table 12 shows the amino acid profiles of chemically treated and extruded products using X-25 (Run IV). High glutamic acid (19.67%), arginine (12.40%), and aspartic acid (9.67%) contents were observed in the raw as well as all treated meals. Except cysteic acid, no noticeable change was observed in the amino acid patterns between the raw material and the treatments, suggesting that there has been a little change in nutritional quality of proteins. Significant reduction in cysteic acid content was noticed in the 2% sodium hydroxide (58% reduction) and sodium bicarbonate (39% reduction) treatments. Control sample (extrusion without chemical treatment) maintained the original cysteic acid content (1.25%) throughout the extrusion process (1.27%). The high cysteic acid

Table 11. Proximate analysis of chemically treated and extruded (Run IV) castor meal[a]

	Raw castor meal	2% NaOH	1% NaOH+ 1% NaOCl	2% $NaHCO_2$	2% $Ca(OH)_2$	Control
Moisture	8.20	2.56	0.89	0.89	5.62	5.62
Protein	37.31	37.44	37.94	40.63	36.75	38.50
Oil	1.75	ND	ND	ND	ND	ND
Crude fiber	30.13	29.17	30.51	31.28	26.32	28.10
Ash	6.91	9.12	10.26	7.94	8.83	6.48

[a] %, as is basis.

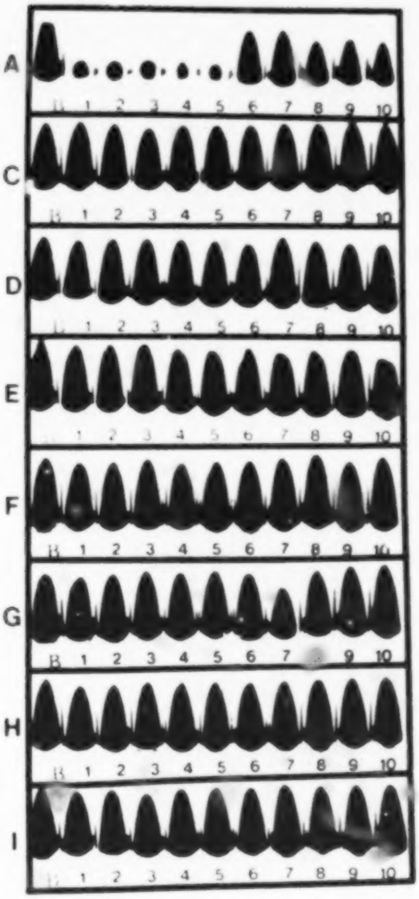


Figure 15. Hemagglutinin reaction of different types of castor bean products. A: Raw castor bean; C: Pre-pressed cake; D-H: Extruded (X-25, Run IV) meal after treatment with 2% NaOH (D), 1% NaOH + 1% NaOCl (E), 2% $NaHCO_3$ (F), 2% $Ca(OH)_2$ (G), and control (H); I: Extruded (X-200, Run V) meal treated with 2% $NaHCO_3$; B: blank; and numbers indicate dilution factors.

Table 12. Amino acid content (%) of chemically treated and extruded castor meal (Run IV)

Amino acid	Raw	2% NaOH	1% NaOH+ 1% NaOCl	2% $NaHCO_3$	2% $Ca(OH)_2$	Control[a]
Lysine	3.53	3.21	4.35	3.46	3.17	3.86
Histidine	2.30	2.27	2.45	2.34	2.13	2.19
Ammonia	2.27	2.67	2.34	2.16	2.16	2.25
Arginine	12.40	11.68	12.97	11.78	11.72	12.21
Aspartic acid	9.67	9.79	9.46	9.66	9.81	9.60
Threonine	3.40	3.27	3.35	3.38	3.34	3.35
Serine	5.65	5.32	5.49	5.67	5.71	5.60
Glutamic acid	19.67	20.34	19.17	19.74	20.18	19.48
Proline	3.40	3.93	3.93	3.83	3.48	4.02
Glycine	4.26	4.4[illegible]	4.21	4.34	4.36	4.22
Alanine	4.34	4.47	4.27	4.43	4.42	4.31
Valine	5.70	5.72	5.00	5.64	5.59	5.53
Methionine	1.74	1.79	1.50	1.73	1.76	1.65
Isoleucine	4.58	4.64	4.48	4.57	4.62	4.48
Leucine	6.43	6.52	6.36	6.47	6.53	6.27
Tyrosine	2.54	1.76	2.39	2.61	2.72	2.65
Phenylalanine	4.04	4.21	3.87	4.15	4.19	4.04
Cysteic acid	1.25	0.57	1.28	0.85	1.31	1.42
Tryptophan	0.50	0.47	0.48	0.67	0.45	0.52
Total	97.67	97.67	97.68	97.66	97.65	97.65

[a] Extruded without chemical treatment.

content in the sample was also reflected to the high residual CB-1A activity, although its contribution to the allergenecity is not known at this time.

VI.4.1.4. *X-200 Run*

Because of the extremely large capacity of the Wenger X-200 extruder, only one chemical treatment (2% sodium bicarbonate) was tested for 1 hr at a throughput of 5,000 Kg/hr to verify the adaptability of the machine for industrial production of the detoxified and deallergenized castor meal. The operational conditions of X-200 were shown in Table 6. Figure 16 shows immunodiffusion test (picture A), the quantitative estimation of the residual CB-1A by dilution technique (picture B), and the shape and size of the product (picture C). Approximately 99% of the CB-1A originally present in the raw castor meal has been destroyed through the chemical treatment (2% sodium bicarbonate) and extrusion as shown in the picture B. Under the test conditions, the extruder ran smoothly, but the pellets did not hold together tightly. The two double conditioning cylinders ran very well for mixing and conditioning the meal, chemicals, and water, thus providing the effective destruction of CB-1A.

VI.4.2. *Treatment of Prepressed Castor Cake*

As an alternative to the detoxification and deallergenization of defatted castor meal, the feasibility of detoxifying and deallergenating the pre-pressed castor cake was evaluated.

Approximately 300 kg of ground pre-pressed castor cakes are treated with the mixture of sodium hydroxide and sodium hypocloride (to a final concentration of 1 % + 1 %, w.w) at a final moisture content of 20 % and extruded using a Wenger X-20 extruder. However, even the combined effect of pre-pressing (Figure 17 B) chemical treatment, and extrusion (Figure 17 C) failed to reduce the residual

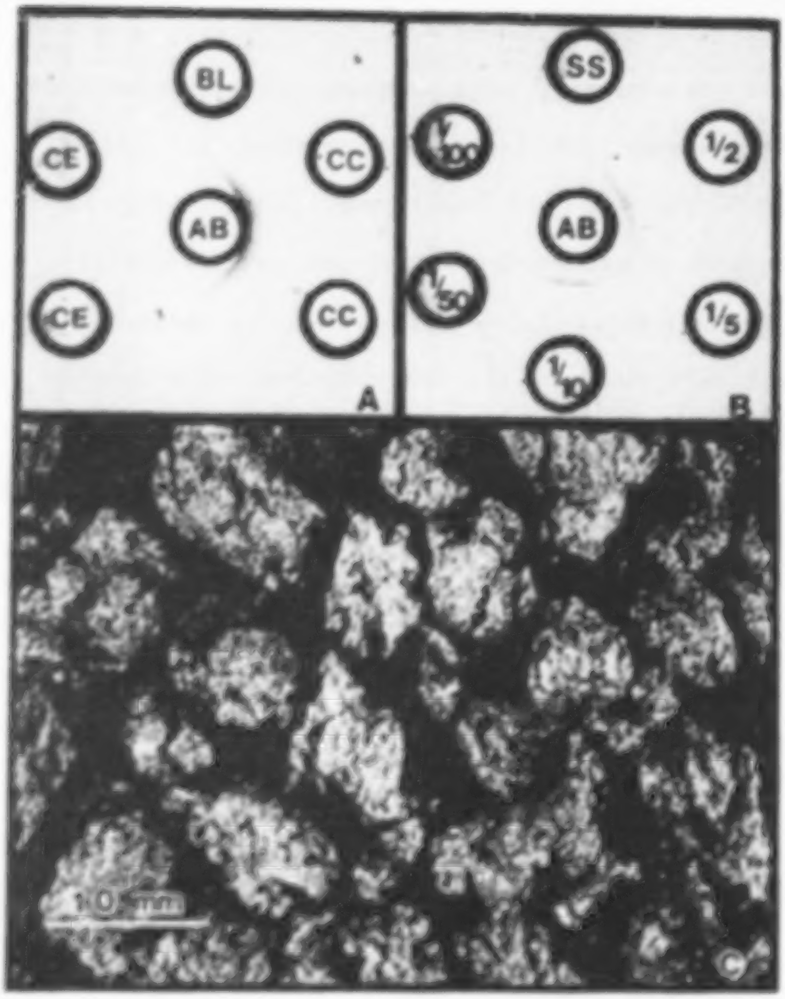

BL
CE
CC
AB
CE
CC
A
SS
1/100
1/2
AB
1/50
1/5
1/10
B
10 mm
C

Figure 16. Immunodiffusion pattern (A), estimation of the relative amount of residual CB-1A by dilution technique (B), and shape and size (C) of the chemically treated and extruded (X-200, Run V) castor meal. AB: antibody; CC: control; BL: blank; CE: sample; 1/2-1/100: dilution factors.

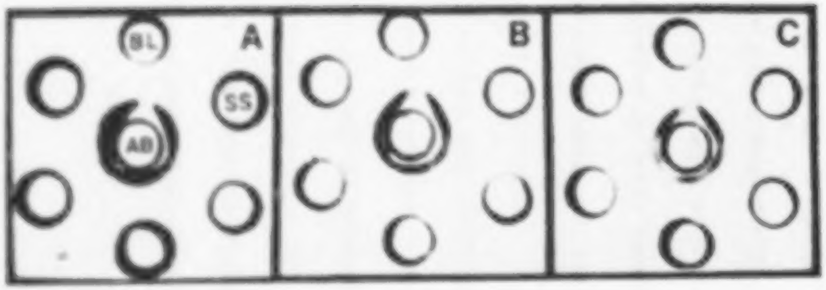


Figure 17. Immunodiffusion patterns for whole castor seed (A), pre-pressed castor cake before (B) and after (C) chemical treatment and extrusion. SS: sample; BL: blank; and AB: antibody.

CB-1A content significantly. As previously described, the identical chemical treatment and extrusion conditions gave almost complete destruction of CB-1A in the defatted meal.

The higher oil content, about 14-15% as measured by the acid hydrolysis oil determination method, might have played an important adverse role in the deallergenation of CB-1A. The oil acted like a barrier among castor cake, steam and chemicals, thus reducing the chance for proper contact among the components, and/or as a lubricant reducing the efficiency of heat generation and transfer. Another technical problem encountered throughout the pre-pressing was the difficulty of reducing the residual oil content to less than 14.5% using a Simons-Rosedowns expeller or Anderson expeller. Expellers were clogged and jammed continuously when attempts were made to tighten the gap to further reduce the oil content of the presscake.

However, the hemagglutinin reaction was absent from the pre-conditioned and pre-pressed castor cake (Figure 15C).

The test results indicate that the detoxification and deallergenation technology developed in this research may not be readily adaptable to the high oil containing pre-pressed cake at this time.

VI.5. Biochemical Evaluation of CB-1A Deallergenation

VI.5.1. *Establishment of a Model System*

It is known that the commercial castor meal usually contains 0.092%-4.2% allergen (Coulson et al. 1960). The routine CB-1A immunodiffusion test procedure used in this experiment involved dissolving 20 g castor meal samples into 50 mL of 0.85% saline solution, thus, in theory, the extract would contain approximately 0.04%-1.68% allergen under ideal conditions. To prepare a model system with similar CB-1A content to this, a 1% CB-1A solution was prepared in phosphate buffer (pH 6.7) using the purified CB-1A powder. To simulate the effect of chemical treatments, calculated amounts of 10% sodium hydroxide and 10% sodium hypochlorite solutions were added to the CB-1A solution to give a final concentration of 250 ppm each, a condition much milder than those employed in the actual deallergenation procedures. The reason for using such a mild condition was based on the possibility that the chemicals in model systems would more freely react with CB-1A, making the deallergenation more effective than in the castor meal. A small amount (1 mL) of the CB-1A solution was then heated by immersing the test tube into a boiling water bath with gentle shaking. The heat-treated (Table 13) solution was immediately cooled down in an ice-bath to avoid an excess heating.

VI.5.2. *Changes in CB-1A Content*

In the model system, the amount of CB-1A decreased drastically as temperature increased (Figure 18. At 50°C and 60°C, no significant reduction of CB-1A has been observed up to 10 min of heating (pictures 50-D and 60-D). But, at 70°C, the amount of CB-1A started to decrease at 3 min heating and became almost undetectable at 30 min. At 80°C, 90°C and 100°C, the destruction of CB-1A was so drastic that the time required for negative immunodiffusion reaction was only 1.5, 1.0, and 0.7 min, respectively.

In contrast to the CB-1A slution that has been chemically treated, a pure CB-1A solution without added chmicals showed an extreme stability against heat. As shown in Figure 19, thick pecipitin lines were still remaining even after 2 hr heating in a boiling water or autoclaving 30 min at 120°C. These results indicate that the role of cheicals is essential for the deallergenation of CB-1A using heat treatment. Te results also show that the heat treatment, to be effective, must be carried out at temperatures above the critical level

Table 13. Conditions used to heat treat CB-1A in model systems

Temp (°C)	Time (min)			
1. 50	1	5	8	10
2. 60	1	5	8	10
3. 70	1	2	3	3.5
4. 80	0.7	1	1.5	-
5. 90	0.3	0.7	1	-
6. 100	0.3	0.7	-	-
7. Control	0	-	-	-

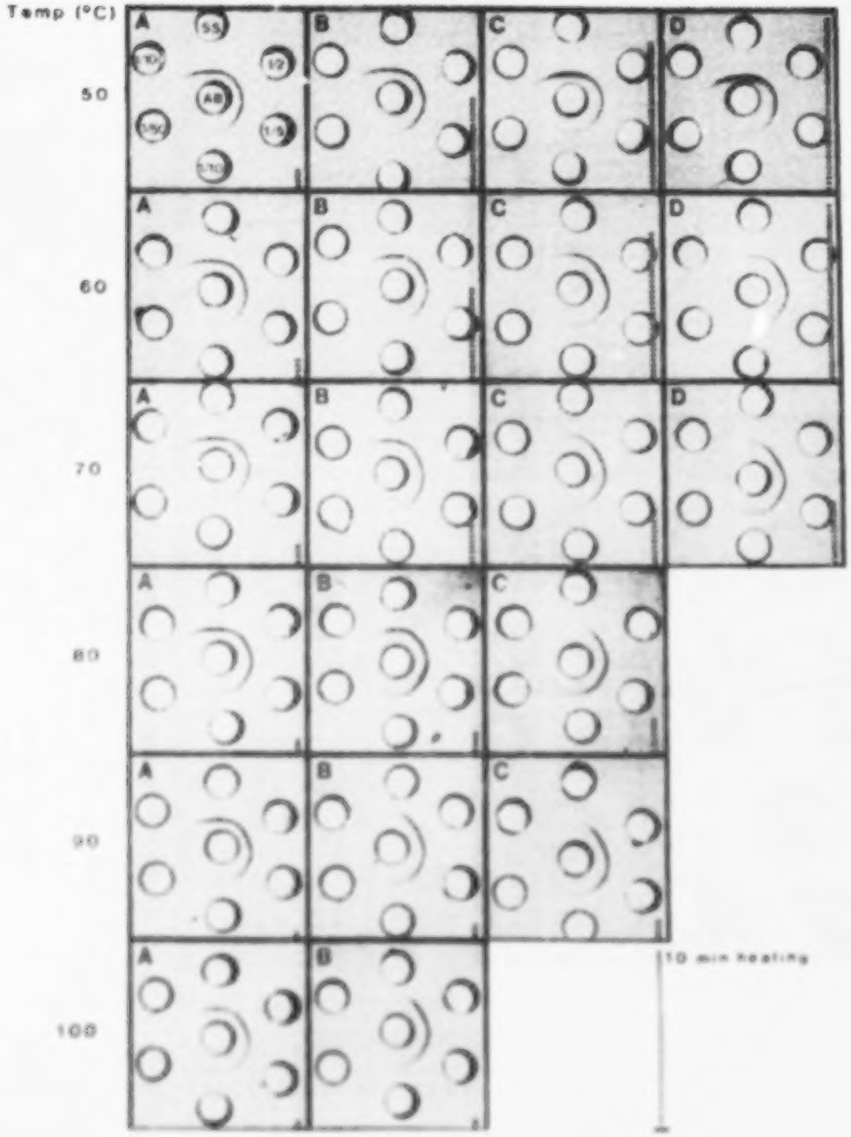

Temp (°C)
50
60
70
80
90
100
A
B
C
D
SS
1/2
1/5
1/10
1/50
1/100
AB
10 min heating

Figure 18. Relative estimation of CB-1A content as affected by heating time and temperature in model systems. AB: antibody; SS: sample; 1/2-1/100: dilution factors. (See Table 13 for heating conditions used.)

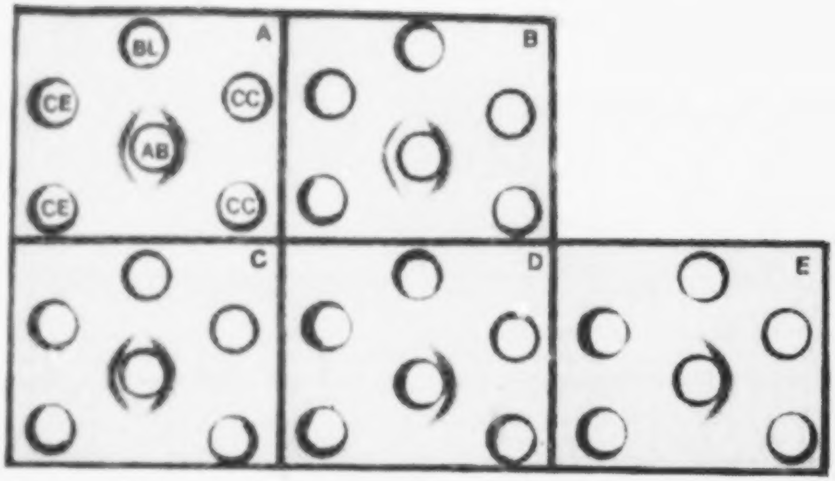


Figure 19. Immunodiffusion pattern of residual CB-1A as affected by heating method and time with (C-E) and without (A-B) chemical treatment in model systems. AB: antibody; BL: blank; CC: control; CE: sample. A: 2 hr heating in boiling water bath; B: 30 min autoclaving at 120°C; C: control; D: 20 sec heating in boiling water bath; E: 40 sec heating in boiling water bath.

(above 70°C in model systems), rather than a longer time at a lower temperature than the critical one.

The destruction of CB-1A activity as expressed by semi-logarithmic plots against heating time at a constant temperature is shown in Figure 20.

VI.5.3. *Disc Gel Electrophoresis*

Disc gel electrophoresis technique is one of the principal tools for characterizing proteins and for assaying their purity. Electrophoretic changes occurred after heat treatment of the 1% aqueous CB-1A solution with or without chemicals are shown in Figure 21. In the control (Sample a), three bands were easily discernible, but traces of one or more bands were also present. These major bands remained principally unaffected after the sample had been placed in boiling water for 2 hr (Sample b). Autoclaving the sample at 120°C for 30 min caused slight diffusion of these bands (Sample d) and formed a new distinct band (band 4 in sample d), but the general pattern has not been greatly altered.

The immunodiffusion precipitin lines were also positively detectable for all heat treated samples without added chemicals (Figure 19, pictures A-B).

In contrast, samples heated with added chemicals showed quite different electrophoretic patterns. The addition of chemicals produced new bands that migrated to regions 35-70 mm in electrophoretic gels (Figure 21, samples e-h). On the other hand, the intensity of existing bands (region 0-35 mm) decreased, with the greatest reduction occurring in the bands at 10-30 mm. At the same time, two more bands (Sample e) appeared in the anodal section (35-45 mm). Furthermore, the appearance of a band at Rf 1.0 (bromophenol blue marker front, samples e-h) became very obvious.

As heating continued after chemical treatment (100°C for 20 sec; Sample f), the intensity of the original bands decreased, which was accompanied by the

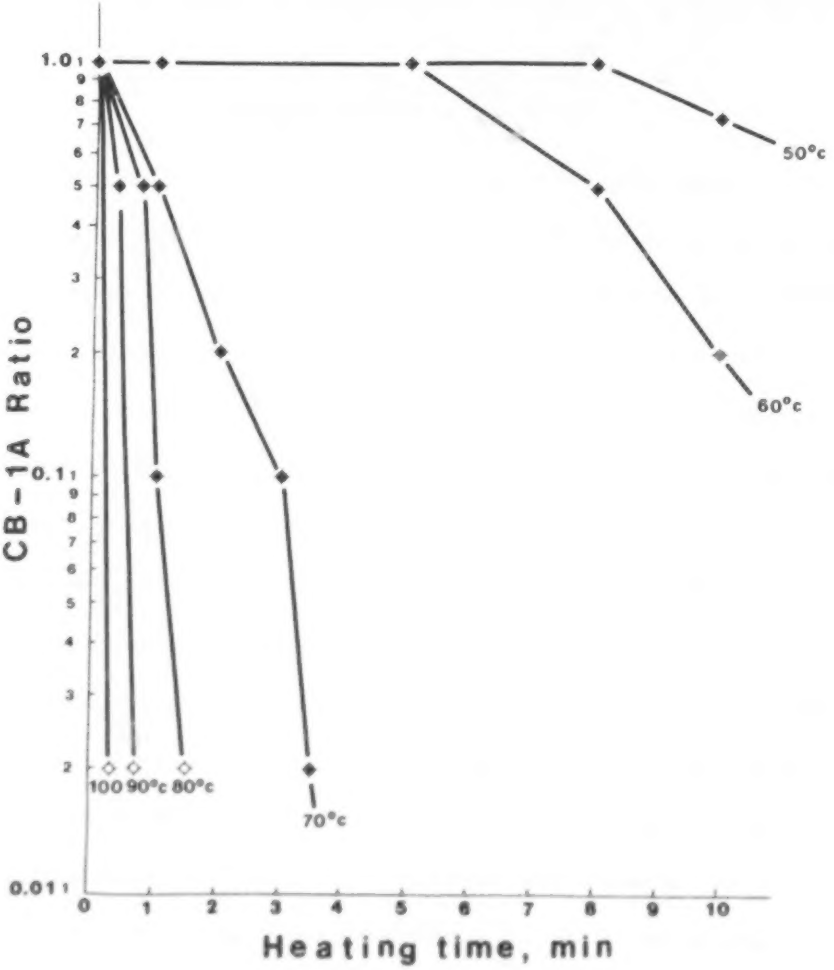


Figure 20. The effect of heating temperature and time on the destruction of CB-1A in model systems.

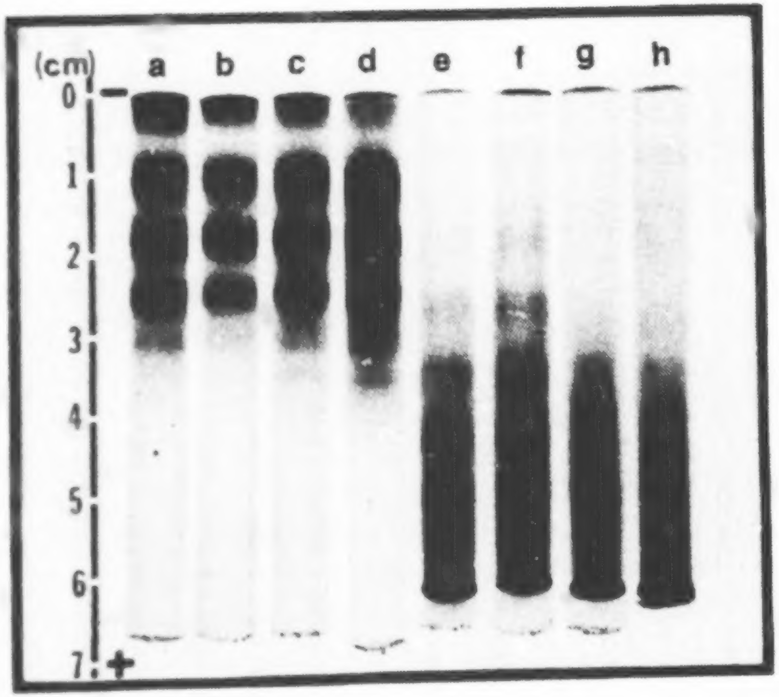


Figure 21. Disc gel electrophoresis pattern of CB-1A as affected by heating time and temperature with (e-h) and without (a-d) added chemicals in model systems. a: control, no heating; b: 2 hr heating in boiling water bath; c: autoclaving at 120°C for 10 min; d: autoclaving at 120°C for 30 min; e: control, no heating; f: 20 sec heating in boiling water bath; g: 40 sec heating in boiling water bath; h: 60 sec heating in boiling water bath.

appearance of additional bands in the region 35-70 mm in Sample f. After heating for 40 sec (Sample g), the bands in the region 10-30 mm became difficult to discern, and at the same time, a broad diffuse band was formed throughout the gels. These changes in banding patterns coinsided with the gradual degradation of antigens (pictures C to E in Figure 19). No immunodiffusion precipitin line was further detected from sample E in Figure 19. The fact that the disappearance of bands in the cathodal section is associated with the simultaneous loss of antigenic property indicates that the bands in the region 0-35 mm are primarily responsible for the allergenecity of CB-1A.

VI.5.4. *SDS Acrylamide Gel Electrophoresis*

Sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis is usually used to resolve and characterize the number and size of protein chains or protein subunits in a protein preparation (Weber and Osborn, 1969). Specifically, purified protein preparations can readily be analyzed for their homogeniety using the SDS electrophoresis technique.

The SDS gel electrophoresis pattern of CB-1A, regardless of chemical and/or heat treatment, showed only a single symmetrical band whose width is consistent with each treatment (Figure 22). The single band first indicates that the isolated CB-1A from defatted castor meal is in a highly purified form. It also indicates that CB-1A is composed of a single polypeptide chain or a group of identical subunits which is not an extremely widespread characteristic of proteins. It is assumed that under the SDS polyacrylamide gel electrophoretic conditions, the electrophoretic velocity is totally dependent on the retardation coefficient (Kr) of the protein which is related only to size and shape (Catsimpoolas, 1977). The same migrated position of the band in each treatment including the control indicates that no dissociation or cleavage of protein

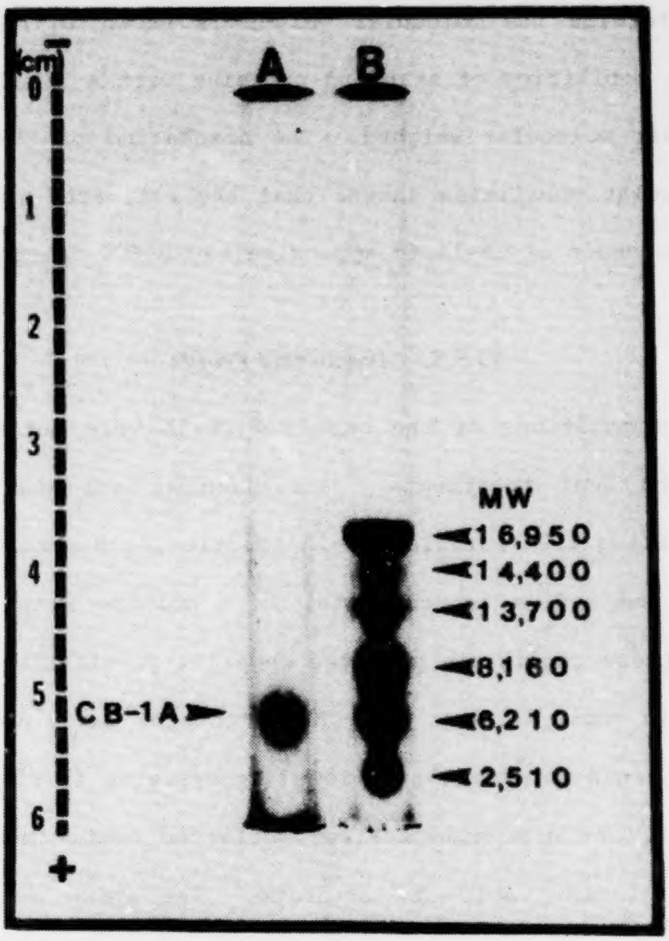

(cm)
−
0
1
2
3
4
5
6
+
A
B
MW
16,950
14,400
13,700
8,160
6,210
2,510
CB-1A

Figure 22. SDS polyacrylamide gel electrophoresis pattern of CB-1A (A) and standard polypeptide mixtures (B) for molecular weight estimation.

molecule has occurred by heating in the presence of sodium hydroxide and sodium hypochlorite.

A linear plot of the standard proteins on which the CB-1A is approximately positioned to determine its molecular weight is shown in Figure 23. It was observed that the mobilities of standard proteins were a linear function of the logarithms of their molecular weights. The bracketing of CB-1A in the linear plot by its molecular mobilities showed that the estimated molecular weight of the single peptide chain of CB-1A is approximately 5,600 daltons.

VI.5.5. *Amino Acid Profile*

Amino acid compositions of the purified CB-1A were determined before and after chemical and heat treatments. The chemical and heat treatments were carried out by heating 1.0% purified CB-1A solution, containing 250 ppm each of sodium hydroxide and sodium hypochlorite, in a boiling water bath for 5 min. Treatments under these conditions produced negative precipitin test.

The results in Table 14 show that CB-1A contains large amounts of glutamic acid (32.78%) and arginine (12.84%) followed by cysteine (7.25% as cysteic acid) and serine (6.75%). As discussed earlier, defatted castor meal also contained large amounts of glutamic acid and arginine. The amino acid composition of CB-1A changed upon chemical and heat treatments. Significant losses of phenylalanine (1.59% to 0%), methionine (1.59% to 0.03%), arginine (12.84% to 9.84%), histidine (1.05% to 0.55%), and cysteine (7.25% to 1.92%) were noticed. However, the relationship between these amino acids and the allergenecity of CB-1A is not known at this time.

VI.5.6. *Sulfhydryl (SH) and Disulfide (SS) Groups*

SH groups, unless masked as in some proteins, are chemically the most active groups found in the cells. SS groups of specific cysteine residues are

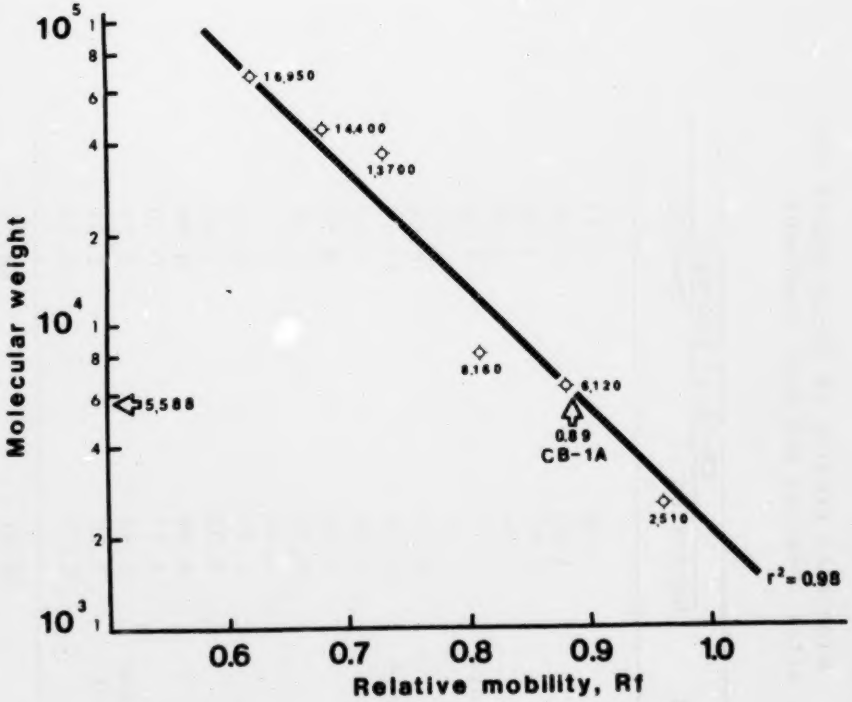


Figure 23. Calibration curve obtained with polypeptides from the commercial molecular weight determination kit. Arrows indicate the molecular weight of CB-1A at its corresponding Rf value in the curve.

Table 14. Amino acid profile of CB-1A before and after chemical and heat treatment

Amino acid	CB-1A (% protein)	
	Untreated	Treated
Lysine	2.90	2.93
Histidine	1.05	0.55
Ammonia	2.65	4.22
Arginine	12.84	9.84
Aspartic acid	3.70	4.56
Threonine	1.02	1.11
Serine	6.75	6.64
Glutamic acid	32.78	37.19
Proline	2.58	2.07
Glycine	3.91	4.57
Alanine	2.36	2.70
Valine	3.32	3.77
Methionine	1.59	0.03
Isoleucine	3.07	3.16
Leucine	4.68	4.98
Tyrosine	1.72	0.20
Phenylalanine	1.59	0.00
Cysteic acid	7.25	1.92
Tryptophan	0.55	0.72
Total	88.51	88.52

important components of the active sites of many enzymes. SH groups are capable of reacting with heavy metals or reagents containing heavy metals (Anglemier and Montgomery, 1976). SS groups are much less active than SH groups and function as stabilizing elements of structure in proteins. However, various reagents can cleave SS bonds and convert them to SH groups or their derivatives (Jocelyn, 1972).

Protein conformation is highly sensitive to minor changes in structure. A minor change in conformation often exerts an appreciable effect on the biological function of a protein (Atassi et al., 1970). For example, trypsin inhibitors became inactive upon SS bond cleavage (Friedman et al., 1982) which often control protein structure (Friedman, 1973).

The SH and SS contents of the purified CB-1A were determined before and after chemical and heat treatments. The chemical and heat treatment conditions were identical to those used in the amino acid profile determination above.

The results in Table 15 show the relative amounts of SH and SS present in CB-1A before and after treatments. The data for ovalbumin are also included as a reference. Surprisingly, almost no SH was detected in CB-1A whether it was treated or not. The data also show that the chemical and heat treatments did not significantly affect the SS content of CB-1A. For ovalbumin, the value for SH was about 4 times larger than that of SS which is in excellent agreement with the published data that ovalbumin contains 4 moles of SH and 1 mole of SS per mole of protein (Beveridge et al., 1974).

At this point, however, the amounts of SH and SS groups present in CB-1A cannot be exactly calculated since additional data on molar absorptivity (or molar extinction coefficient) of the purified CB-1A is needed along with its more accurate molecular weight. Research involving these topics will continue in the future.

Table 15. Sulfhydryl and disulfide contents of CB-1A before and after chemical and heat treatments

	SH (A_{412})	SS (A_{412})
CB-1A		
Control	0.006	0.711
Treated	0.012	0.646
Ovalbumin	0.731	0.194

VI.5.7. *Rocket Immunoelectrophoresis*

Rocket immunoelectrophoresis is a technique for the quantitative analysis of a single antigen in a complex mixture of proteins. The protein sample is placed in a well and moved by electrophoresis into a gel containing evenly distributed antibodies. Migration continues until the antigen is diluted enough to form a tip of the rocket shaped precipitin peak whose height is proportional to the concentration of the antigen (Axelsen and Svendsen, 1973).

As a quantification measure of CB-1A in raw castor meal, a rocket immunoelectrophoresis was employed using a known amount of purified CB-1A solution (0.1%) as a standard as shown in Figures 24 and 25 and tabulated in Table 15. A strong linear co-relation exists between the peak height and the concentration of antigen (r^2=0.98, Figure 25). The estimated amount of CB-1A in 10 μL of unknown sample solution in full strength was equivalent to 19.6 μL of 0.1% purified CB-1A solution (Table 16). Since the ratio of saline solution (50 mL) to castor meal (20 g) was 2.5:1 (v/w), 10 μL (=0.01 mL) of the crude CB-1A extract contained the same amount of CB-1A as 0.004 g (0.01 x 1/2.5) of raw castor meal. Accordingly, 100 g castor meal contains the equivalent amount of CB-1A to 4.9 x 10^5 μL of 0.1% CB-1A solution. Another word, 0.49 g pure CB-1A exists in 100 g castor meal. Assuming the specific gravity of crude CB-1A extract is 1.0 and 100% of CB-1A in the castor meal was recovered into saline solution extract, the CB-1A content of the commercial castor meal is approximately 0.49% (w/w). The rocket immunoelectrophoresis pattern seems to indicate that the minimum amount of CB-1A detectable by rocket immunoelectrophoresis is about 4 x 10^{-4} g (Figure 24 and Table 16).

As compared to the dilution technique where only a broad range of dilution ratio was applicable, stepwise measurement of the peak height of the rocket immunoelectrophoresis was possible which proportionally corresponds to the antigen concentration. However, the indistinctiveness of the top of the peak

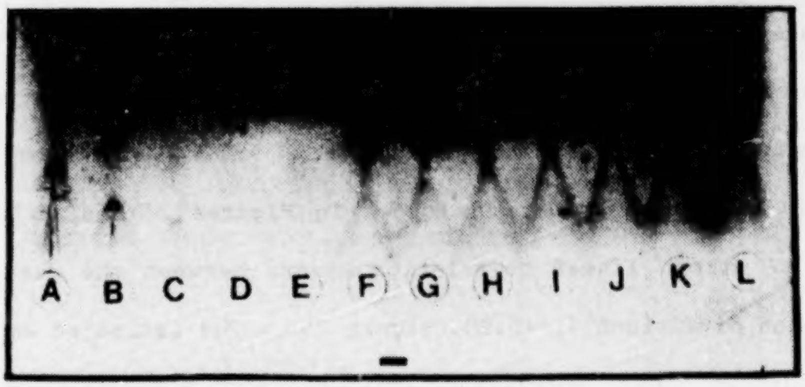


Figure 24. Quantification of CB-1A in raw castor meal by rocket immunoelectrophoresis. A, B, C, D, and E: 1/2, 1/5, 1/10, 1/50 and 1/100 dilutions of unknown sample; F, G, H, I, J, K, and L: 4, 5, 6, 7, 8, 9 and 10 ul of 0.1% CB-1A standard solution.

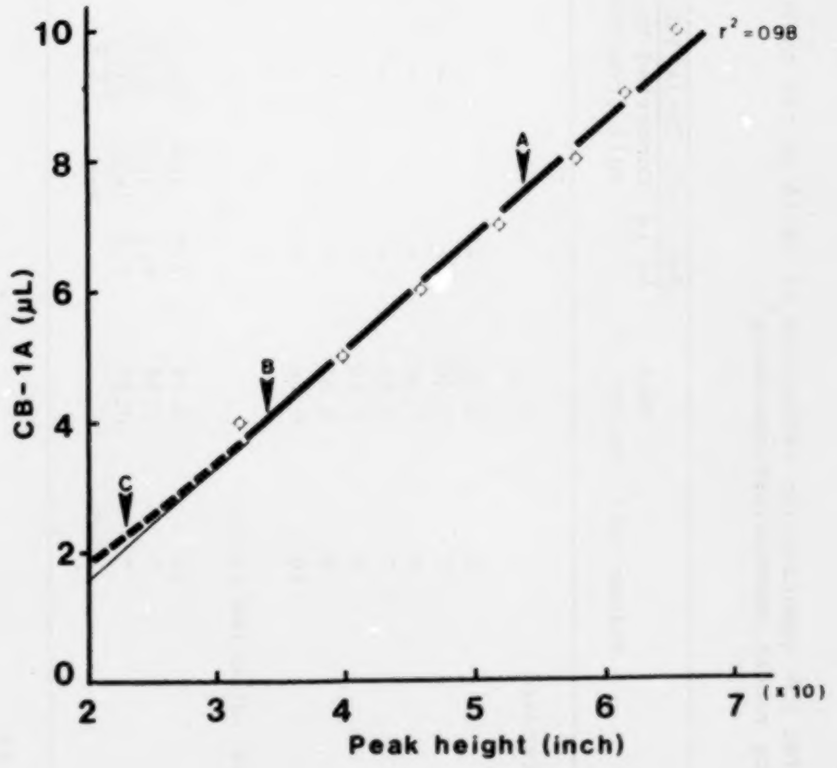


Figure 25. Calibration curve obtained with different volume of purified CB-1A solution and bracketed unknowns. A, B and C: 1/2, 1/5, and 1/10 dilutions of unknown.

Table 16. Data for the quantitative estimation of CB-1A in raw castor meal by rocket immunoelectrophoresis[a]

Hole number	volume (uL)	Peak height (")	Est. vol. CB-1A (uL) As is		Converted to full strength
1. 0.1% CB-1A standard					
F	4	0.32	-		-
G	5	0.40	-		-
H	6	0.46	-		-
I	7	0.52	-		-
J	8	0.58	-		
K	9	0.62	-		
L	10	0.66	-		
2. Unknown sample (dilution ratio)					
A (1:2)	10	0.54	7.6	(x2)	15.2
B (1:5)	"	0.34	4.1	(x5)	20.5
C (1:10)	"	0.23	2.3	(x10)	23.0
				Avg.	19.6

---

[a] Refer to Figure 24.

with its long tails made it difficult to measure the exact height of the peak. The long developing time (approximately 5-7 hr) of the electrophoresis, the need for a large amount of antibody (0.3 mL per 10 x 10 cm plate), and the uneven migration of antibody in the gel during electrophoresis when the isoelectric point of antibody is not exactly identical to the buffer pH of the gel are still considered the obstacles of this method. The time-consuming staining, destaining and drying procedures of the gel are the additional factors to be considered.

VI.6. Animal Feeding Tests

VI.6.1. *Rat Feeding Tests*

Table 17 shows the result of the protein efficiency ratio (PER) studies. The proximate analysis data for the reference casein and the test samples are shown in Table 18. Clearly, the PER values of all castor meal samples were considerably lower than the casein standard. Also, the average body weight gained and the average amount of protein consumed by the rats on castor meal diets were considerably lower than those on casein diet. These results probably mean that castor meal may not be recommended for use as the sole source of protein, at least for rats, and perhaps for other animals also.

VI.6.2. *Chick Feeding Tests*

VI.6.2.1. *Battery Brooder Study (Experiment I)*

There were not significant treatment differences in body weigh and feed conversion (Table 19). However, there were non-significant numerical differences, suggesting a possible progressive improvement of both growth and feed conversion with each processing method from NaOH treated castor meal (Diet 2) to NaOH+NaOCl treated one (Diet 5).

Table 17. Data[a] for the PER study conducted on the detoxified and deallergenized castor meal

Sample Description	Average body weight gain, g	Average protein consumed, g	PER	
			As is	Corrected
Diet 0, Control[b]	157.50 (5.68)	52.90 (2.09)	2.98	2.50
Diet 1, Untreated	26.50 (4.71)	28.37 (6.69)	0.88	0.74
Diet 2, 2% NaOH	42.90 (3.62)	40.48 (7.23)	1.04	0.88
Diet 3, 2% $NaHCO_3$	17.40 (3.16)	29.68 (7.17)	0.56	0.47
Diet 4, 2% $Ca(OH)_2$	34.60 (6.44)	34.16 (11.77)	0.93	0.78
Diet 5, 1% NaOH + 1% NaOCl	24.20 (3.25)	30.98 (6.79)	0.75	0.63

[a] Numbers in parenthesis indicate the values for the standard error.

[b] ANRC casein Lot # 4NH08S13.

Table 18. Composition[a] of diets for protein efficiency ratio determination

Sample	Protein	Fat	Fiber	Ash	Moisture
Diet 0, Control[b]	92.0	1.6	0	1.2	3.0
Diet 1, Untreated	37.3	1.8	30.0	6.9	8.2
Diet 2, 2% NaOH	37.4	1.8	29.2	9.1	2.6
Diet 3, 2% $NaHCO_3$	40.6	1.8	31.3	7.9	0.9
Diet 4, 2% $Ca(OH)_2$	36.8	1.8	26.3	8.8	5.6
Diet 5, 1% NaOH + 1% NaOCl	37.9	1.8	30.5	10.3	0.9

[a] %, as is basis.

[b] ANRC casein Lot #4NHO8S13.

Table 19. **Effects of Detoxified and Deallergenized Castor Meal Products on Mortality, Body weight, and Feed Conversion in Three Week Old Broilers (Experiment I)**

Treatment	Level	Mortality	Week	Body Weight,g	Feed Conversion
[Diet 0: Soy Bean Meal, Control]					
	0%	0	1	111.6 (1.1)	1.24 (0.01)
	0%	0	2	271.7 (2.5)	1.42 (0.01)
	0%	1	3	500.6 (14.0)	1.58 (0.05)
[Diet 1: Untreated Castor Meal]					
	4%	0	1	107.4 (3.8)	1.24 (0.03)
	4%	1	2	262.5 (7.2)	1.46 (0.04)
	4%	0	3	496.4 (17.2)	1.49 (0.06)
	8%	0	1	106.1 (7.0)	1.25 (0.02)
	8%	1	2	243.2 (3.6)	1.55 (0.05)
	8%	0	3	436.5 (33.0)	1.78 (0.16)
	12%	2	1	105.3 (5.2)	1.46 (0.11)
	12%	2	2	243.0 (13.1)	1.63 (0.09)
	12%	0	3	453.4 (39.2)	1.82 (0.14)
[Diet 2: 2% NaOH Treatment]					
	4%	0	1	111.7 (4.2)	1.24 (0.04)
	4%	1	2	242.9 (39.8)	1.73 (0.29)
	4%	0	3	457.1 (55.7)	1.76 (0.11)
	8%	2	1	104.5 (6.1)	1.34 (0.05)
	8%	0	2	265.8 (7.3)	1.47 (0.02)
	8%	0	3	494.1 (10.0)	1.58 (0.06)
	12%	0	1	108.2 (5.9)	1.31 (0.04)
	12%	0	2	249.5 (14.2)	1.63 (0.08)
	12%	0	3	483.0 (12.3)	1.67 (0.13)

(Table 19 continued)

Treatment	Level	Mortality	Week	Body Weight,g	Feed Conversion
(Diet 3: 2% $NaHCO_3$ Treatment)					
	4%	2	1	106.7 (4.6)	1.37 (0.07)
	4%	1	2	263.6 (12.2)	1.52 (0.06)
	4%	0	3	477.3 (53.8)	1.72 (0.12)
	8%	0	1	99.1 (3.2)	1.36 (0.07)
	8%	1	2	255.8 (6.9)	1.51 (0.08)
	8%	0	3	505.9 (10.8)	1.59 (0.06)
	12%	0	1	103.4 (8.7)	1.33 (0.03)
	12%	0	2	255.2 (13.8)	1.49 (0.02)
	12%	1	3	497.2 (22.3)	1.58 (0.14)
(Diet 4: 2% $Ca(OH)_2$ Treatment)					
	4%	0	1	120.5 (3.7)	1.26 (0.01)
	4%	0	2	266.6 (8.4)	1.51 (0.03)
	4%	0	3	517.6 (12.4)	1.57 (0.04)
	8%	1	1	112.9 (2.6)	1.35 (0.09)
	8%	1	2	278.2 (4.7)	1.50 (0.02)
	8%	0	3	517.1 (10.4)	1.64 (0.03)
	12%	1	1	97.7 (3.8)	1.32 (0.06)
	12%	0	2	237.5 (6.7)	1.55 (0.05)
	12%	0	3	475.5 (7.7)	1.59 (0.07)
(Diet 5: 1% NaOH + 1% NaOCl Treatment)					
	4%	1	1	111.7 (5.7)	1.33 (0.04)
	4%	0	2	276.0 (8.7)	1.50 (0.03)
	4%	0	3	522.0 (22.1)	1.53 (0.08)
	8%	0	1	118.5 (3.2)	1.29 (0.02)
	8%	1	2	275.4 (7.9)	1.43 (0.04)
	8%	0	3	506.6 (14.1)	1.58 (0.03)
	12%	1	1	109.1 (3.0)	1.31 (0.03)
	12%	0	2	260.3 (6.3)	1.56 (0.05)
	12%	1	3	501.0 (12.9)	1.65 (0.08)

[a] **Each value is the Mean (Standard Error) of 40 determinations.**

The level of castor meal in the diet did not significantly affect either growth or feed conversion, however birds receiving the lower level of castor meal seemed to perform better.

Mortality appeared to be random and was not associated with any particular treatment.

Histopathological examination of selected tissues from the control group and birds fed 12% castor meal were non-conclusive (Table 20).

VI.6.2.2. *Floor Pen Study (Experiment II)*

At day 29 of the experiment there were not significant differences in either average body weight or feed conversion (Table 21). By 43 days of age, however, birds receiving Diet 1 (untreated castor meal) weighed significantly less ($p<0.05$) than birds receiving any of the other diets (Table 21).

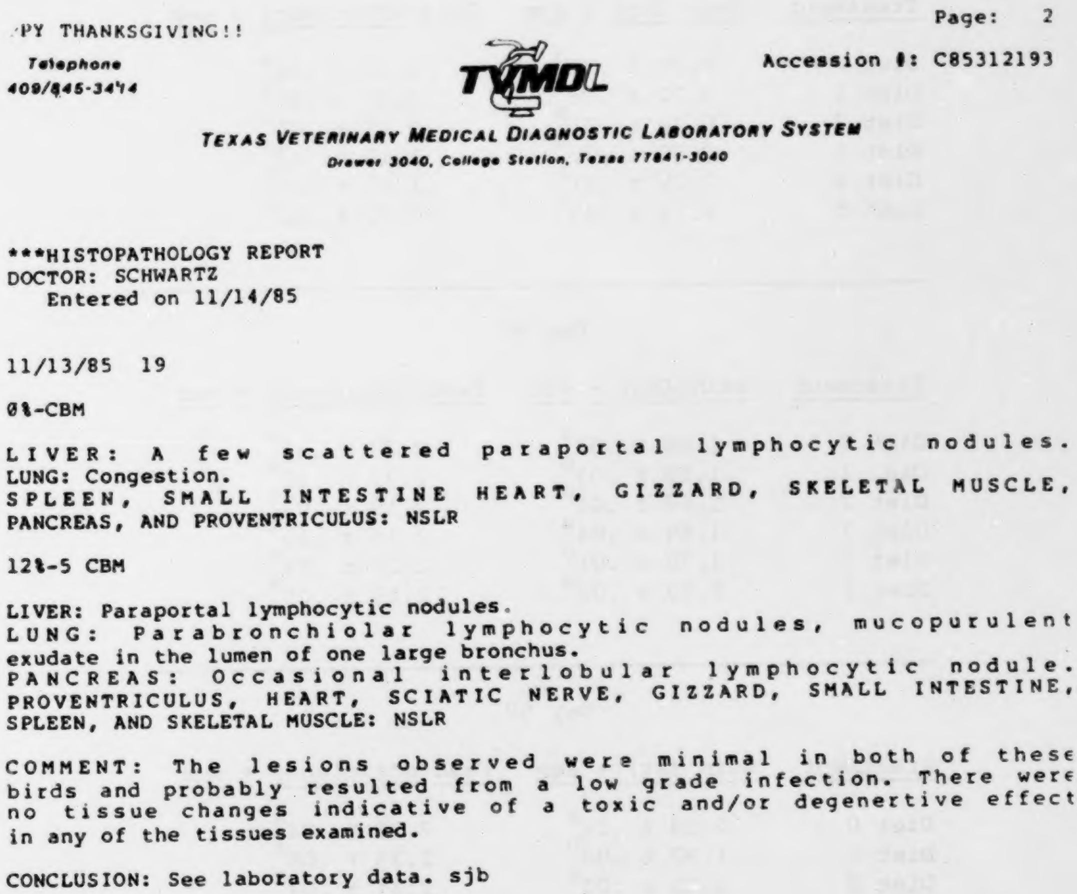
At day 43, all the treated castor meal diets resulted in higher average weight gains than the control diet.

At day 50, birds receiving the untreated castor meal (Diet 1) again weighed significantly less ($p<0.05$) than those receiving any of the other diets (Table 21). Birds receiving Diet 5 weighed the most, averaging 2.17 kg per bird.

Mortality was not considered significant in this study. The birds ate more feed than expected probably because of the cold weather during the study. Temperatures as low as -2.2°C were recorded inside the broiler house on February 11th and 12th, 1986. Most of the mortality occurred during this time.

Histopathology was done at the Texas A&M University Veterinary Diagnostics Lab. There were no significant differences between any of the slides examined (Table 22).

Table 20. Histopathological report on selected organs collected from battery brooders



PY THANKSGIVING!!

Page: 2

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409/845-3414

TVMDL

Accession #: C85312193

TEXAS VETERINARY MEDICAL DIAGNOSTIC LABORATORY SYSTEM
Drawer 3040, College Station, Texas 77841-3040

***HISTOPATHOLOGY REPORT
DOCTOR: SCHWARTZ
Entered on 11/14/85

11/13/85 19

0%-CBM

LIVER: A few scattered paraportal lymphocytic nodules.
LUNG: Congestion.
SPLEEN, SMALL INTESTINE HEART, GIZZARD, SKELETAL MUSCLE, PANCREAS, AND PROVENTRICULUS: NSLR

12%-5 CBM

LIVER: Paraportal lymphocytic nodules.
LUNG: Parabronchiolar lymphocytic nodules, mucopurulent exudate in the lumen of one large bronchus.
PANCREAS: Occasional interlobular lymphocytic nodule.
PROVENTRICULUS, HEART, SCIATIC NERVE, GIZZARD, SMALL INTESTINE, SPLEEN, AND SKELETAL MUSCLE: NSLR

COMMENT: The lesions observed were minimal in both of these birds and probably resulted from a low grade infection. There were no tissue changes indicative of a toxic and/or degenertive effect in any of the tissues examined.

CONCLUSION: See laboratory data. sjb

Table 21. Effects of various castorbean meal treatments on weight gain and feed efficiency in 29, 43 and 50 day old male broilers

Day 29

Treatment	Gain (kg) ± sem	Feed efficiency ± sem
Diet 0	0.81 ± .01^{a}	2.02 ± .06^{a}
Diet 1	0.72 ± .08^{a}	2.33 ± .30^{a}
Diet 2	0.79 ± .01^{a}	2.11 ± .05^{a}
Diet 3	0.80 ± .02^{a}	2.10 ± .12^{a}
Diet 4	0.79 ± .01^{a}	1.94 ± .15^{a}
Diet 5	0.79 ± .03^{a}	2.01 ± .02^{a}

Day 43

Treatment	Gain (kg) ± sem	Feed efficiency ± sem
Diet 0	1.66 ± .02^{a}	2.27 ± .04^{a}
Diet 1	1.58 ± .03^{b}	2.32 ± .10^{a}
Diet 2	1.69 ± .01^{a}	2.19 ± .03^{a}
Diet 3	1.69 ± .04^{a}	2.19 ± .20^{a}
Diet 4	1.70 ± .01^{a}	2.27 ± .04^{a}
Diet 5	1.72 ± .03^{a}	2.18 ± .05^{a}

Day 50

Treatment	Gain (kg) ± sem	Feed efficiency ± sem
Diet 0	2.14 ± .02^{a}	2.21 ± .04^{a}
Diet 1	1.97 ± .03^{b}	2.39 ± .06^{a}
Diet 2	2.11 ± .03^{a}	2.31 ± .03^{a}
Diet 3	2.11 ± .04^{a}	2.37 ± .06^{a}
Diet 4	2.10 ± .03^{a}	2.32 ± .04^{a}
Diet 5	2.17 ± .04^{a}	2.30 ± .04^{a}

^{a,b} Means within a column with same superscripts are not significantly different ($p<.05$).

Table 22. Histopathology report on selected organs collected from market broiler hens

Histopathology Report
Doctor Jones
Entered on 4/11/86

04/11/86 35 slides

CONTROL:
LIVER: Many lymphocytic foci especially around bile ducts.
KIDNEY, SPLEEN, PANCREAS, PROVENTRICULUS, SMALL INTESTINE, LUNG, BRAIN,HEART, SKELETAL MUSCLE, CROP, LARGE INTESTINE: NSLR.

BIRD 1-3:
LIVER: Lymphatic accumulations also around bile ducts.
KIDNEY, SPLEEN, PANCREAS, PROVENTRICULUS, SMALL INTESTINE, LUNG, BRAIN,HEART, SKELETAL MUSCLE, CROP, LARGE INTESTINE: NSLR.
HEART: Vacuolar degeneration of some myofibers.

BIRD 2-3:
LIVER: Lymphocytic foci.
GIZZARD: Focal degeneration and inflammatory cells response within the smooth muscle of the Gizzard.
HEART: Mild vacuolar degenerative lesions within the myofibers.
SPLEEN, PANCREAS, PROVENTRICULUS, SMALL INTESTINE, BRAIN, SKELETAL MUSCLE, LARGE INTESTINE: NSLR.

BIRD 3-2:
BRAIN: Occasional perivascular cuff.
HEART: Focal degeneration of mycardium with lipid accumulation between fibers.
BREAST MUSCLE: An area of myodegeneration and lipid accumulation.

No lesions were found in liver, kidney, spleen, pancreas, proventriculus, small intestine, lungs, crop, and large intestine.

BIRD 4-3:
LIVER: Lymphocytic foci around bile ducts.
HEART: Mild focal myodegeneration.
SKELETAL MUSCLE: Mild focal myodegeneration.

No lesions were found in the kidney, spleen, pancreas, proventriculus, small intestine, lungs, crop, and large intestine.

BIRD 5-2:
SPLEEN: Some necrosis of the lymphoid tissue.
GIZZARD: Very mild focal degeneration of the muscle wall.
HEART: There is a very mild focal myodegeneration.
SKELETAL MUSCLE: Very mild focal myodegeneration.

No lesions were found in liver, kidney, pancreas, proventriculus, small intestine, lung, brain and crop. In some birds, some tissues were not present in control Bird: no gizzard. In Bird 2-3: no lung was found and no crop was found. In Bird 3-2: no gizzard was found. In Bird 4-2: no gizzard was found and no lung was found.

CONCLUSION: Experimental tissues as listed. /agm

VI.6.2.3. *Laying Hens Study (Experiment III)*

There were significant differences ($p<0.05$) in interior egg quality noticeable as early as one week on the castor meal treatments. Parameters affected include albumin height, albumin diameter, and yolk color as determined by La-Rouche color fan.

By the second week, significant difference in total egg production ($p<0.10$) and egg weight ($p<0.05$) became apparent. Birds receiving Diet 5 (1% NaOH + 1% NaOCl treated castor meal) performed the best while hens receiving Diet 1 (untreated castor meal) performed the poorest.

By the sixth week of the study, significant effects ($p<0.10$) were apparent for albumin height, albumin diameter, color, and hen day production (Figure 26). Complete data covering the entire feeding period and Duncan's Multiple Comparison Test of the data are presented in Appendices I and II.

Although egg production was not detrimentally affected by up to 10% treated castor meal, interior egg quality seemed to deteriorate.

Mortality was not considered a factor in this study as only two hens died, one from the control group and one from NaOH treated castor meal group.

VI.6.3. *Swine Feeding Tests*

There were no symptoms of all health among the pigs during the trial. The greatest observed difference in physical appearance among the pigs was the gauntness of pigs fed Diet 1 (Untreated) at the beginning of the trial. Only one pig (from Diet 4) was removed from the test, and that was due to a foot injury.

A summary of the histological observations is shown in Appendix III. There appears to be no correlation between the observations and the dietary treatments. It has been indicated that none of the pigs had severe enough lesions to be diagnosed clinically ill.

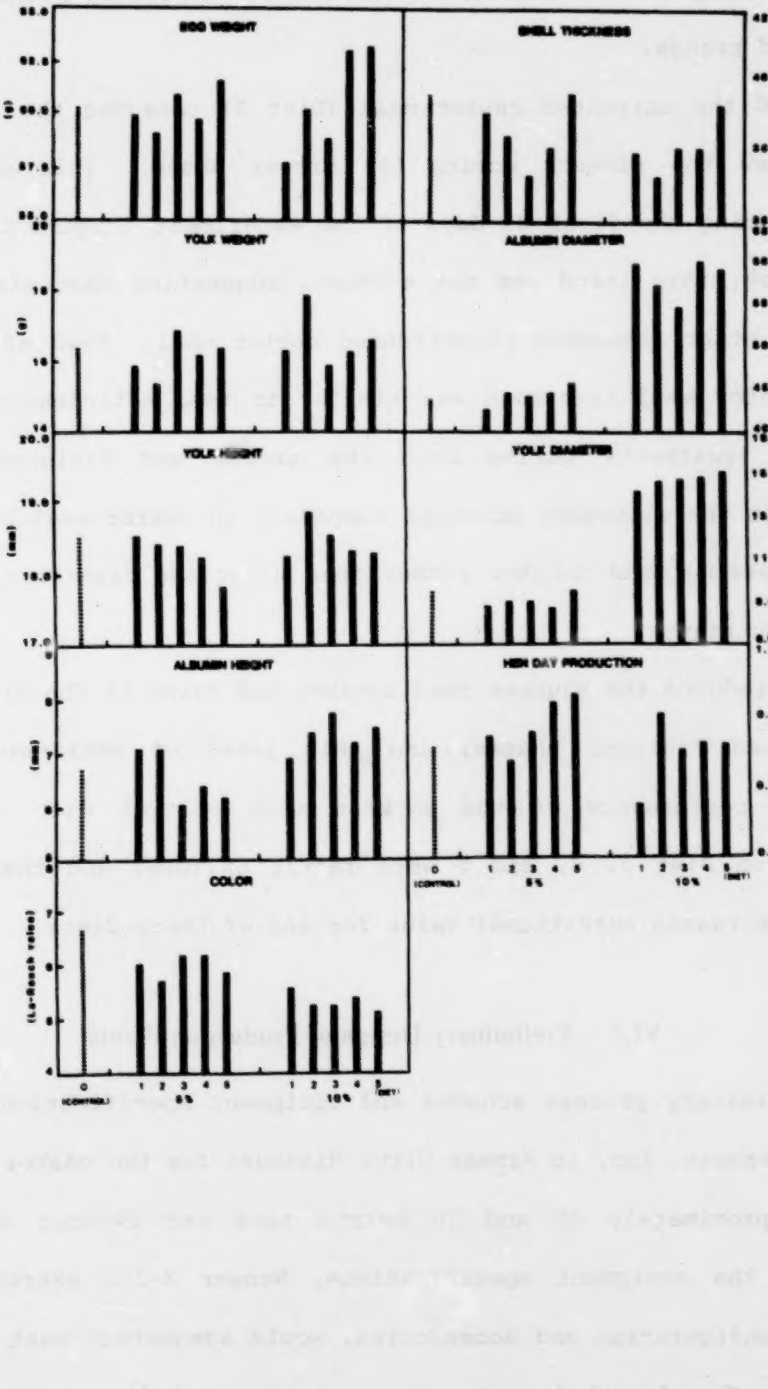

Figure 26. Internal egg quality parameters at the end of the 6th week of study.

Average pig performance during the grower and finisher phase and over the total trial are shown in Table 23. There were not clear cut statistical differences among the treatments, so the results discussed below deal primarily with observed trends.

Pigs fed the untreated castor meal (Diet 1) consumed the least amount of feed and grew the slowest during the grower phase. This effect was most noticeable during the first 33 days of the experiment (Figure 27). During the finisher phase, this trend was not evident, suggesting that pigs can adapt to allergens or other compounds in untreated castor meal. Feed efficiency on the untreated castor meal treatment was similar to feed efficiencies on the other castor meal treatments during both the grower and finisher phase. This indicates that the allergens or other compounds in castor meal affect growth of pigs by supressing feed intake, rather than affecting digestion or utilization of ingested nutrients.

Diet 2 produced the highest feed intakes and gains of the diets during both the grower and finisher phases; and this level of performance equaled or exceeded the performance on the soybean meal control diet (Diet 0). Pig performances on Diet 3, 4, and 5 were fairly uniform, and there was not any evidence of decreased nutritional value for any of these diets.

VI.7. Preliminary Design of Production Plants

The preliminary process schemes and equipment specifications were made by the Wenger Overseas, Inc. in Kansas City, Missouri for two castor meal treatment plants of approximately 25 and 50 metric tons per 24-hour day capacities. According to the equipment specifications, Wenger X-175 extruders, with the recommended configuration and accessories, would adequately meet all production requirements. The detailed equipment proposal, including cost estimates, are presented in its entirety in Appendix IV for future reference.

Table 23. Performance of pigs fed diets containing differently processed castorbean meals on soybean meal[a]

	Diet 0	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	CV
Grower phase							
Initial weight, kg	23.0	82.9	23.0	22.6	23.0	23.1	
Average daily gain, kg	$.70^{c}$	$.83^{b}$	$.77^{bc}$	$.78^{bc}$	$.80^{b}$	$.84^{b}$	8.1
Average daily feed, kg	2.00^{c}	2.33^{b}	2.21^{bc}	2.16^{bc}	2.38^{bc}	2.28^{bc}	4.9
Gain/feed	.35	.36	.35	.36	.34	.37	6.0
Final weight, kg	55.8	61.8	59.3	59.1	60.8		
Finisher phase							
Average daily gain, kg	.88	.98	.90	.88	.90	.87	8.6
Average daily feed, kg	3.03^{bc}	3.32^{b}	3.11^{bc}	2.97^{c}	3.13^{bc}	2.97^{c}	6.8
Feed/gain	.29	.30	.29	.30	.29	.29	6.6
Final weight, kg	102.2	109.1	103.2	101.4	103.6	105.1	
Total trial							
Average daily gain, kg	$.79^{c}$	$.91^{b}$	$.84^{bc}$	$.83^{bc}$	$.85^{bc}$	$.86^{bc}$	7.2
Average daily feed, kg	2.55^{c}	2.83^{b}	2.67^{bc}	2.56^{bc}	2.75^{bc}	2.64^{bc}	6.9
Gain/feed	.31	.32	.32	.32	.31	.33	5.3

[a] Values are means for 10 pigs (2/pen) except values for castorbean meal treatment 342 which are means for 9 pigs.

[bc] $P<.05$.

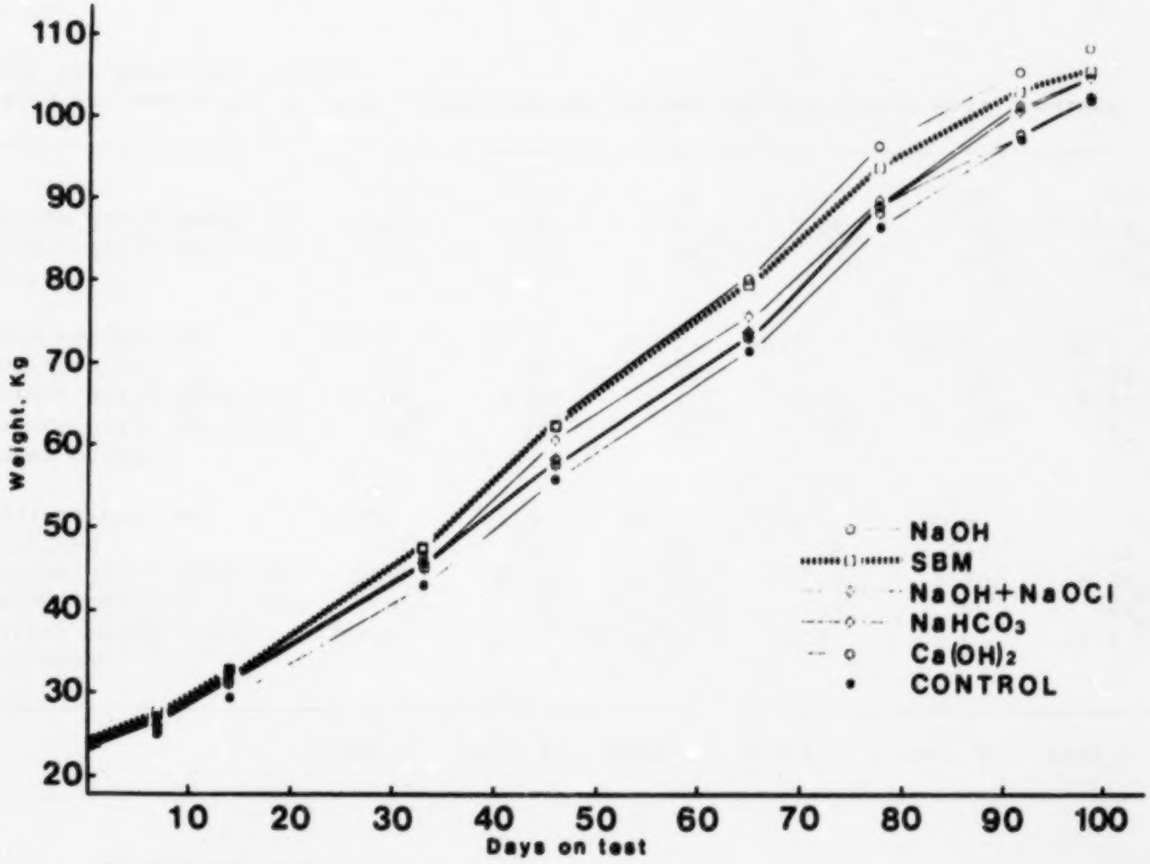

Figure 27. Accumulative average weights of pigs fed various castormeal and soybean meal.

VII. CONCLUSIONS

Based on the combined results obtained from Phase II and Phase III studies, the following conclusions are made.

1. Ricin is completely destroyed during pre-pressing of castor seeds and/or desolventization of solvent extracted meals.
2. The hemagglutin in reaction technique is relatively simple, sensitive, and reproducible to be adapted as a routine analytical tool for ricin toxicity testing.
3. Sodium hydroxide-sodium hypochlorite mixture, calcium hydroxide, sodium bicarbonate, sodium hydroxide, and sodium hypochlorite, in the order of preferrence, are very effective in destroying CB-1A when used in combination with proper heat treatment. However, chemical treatment alone is not as effective in destroying CB-1A.
4. A significant amount, as much as 90% of the residual CB-1A, is destroyed at total chemical concentrations of 1.5%. At 2.0% level, the destruction is almost complete (more than 98%).
5. Extruders are very effective high temperature-short time chemical reactors for destruction of CB-1A if used with proper chemicals. Extrusion alone, however, is not as effective for CB-1A destruction.
6. To be effective, the extrusion temperature should reach at least 130°C, but preferably around 150°C.
7. Proper mixing of chemicals with the meal is of paramount importance for effective destruction of CB-1A.
8. Extrusion processing of chemically treated castor meal is readily adaptable for scaled-up commercial production of detoxified and deallergenated castor meal as demonstrated by Wenger X-20, X-25, and X-200 runs.

9. The detoxified and deallergenated castor meal is safe for use as animal feeds as demonstrated by chick and swine feeding studies.
10. Both the immunodiffusion technique and the dilution technique in conjunction with the immunodiffusion technique are somewhat time-consuming, but these methods are extremely sensitive, specific, and reproducible for qualitative and quantitative determinations of CB-1A, respectively, to be used as standard quality control methods.
11. The rocket immunoelectrophoresis can determine the amount of CB-1A very accurately, but it is very difficult and time-consuming to master the technique for use in routine assays.
12. The low PER (Protein Efficiency Ratio) values obtained from rat feeding studies indicate that the treated castor meal may not be used as the sole source of dietary proteins for animals.

VIII. RECOMMENDATIONS

The experimental results thus far obtained from Phase I, Phase II, and Phase III studies strongly suggest that the technology for commercial production of detoxified and deallergenated castor meal for use in animal feed is now available for implementation. The castor bean industry is therefore advised to consider the following recommendations to implement the new technology with UNIDO assistance.

1. Analyze the economics of implementing this new technology for the existing castor bean processing facilities under prevailing conditions on a care-by-case.
2. Determine the market potential for the new product.
3. Set up demonstratoin and/or production plants using the new technology at current castor seed processing facilities.

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APPENDIX I

Effect of Chemically Treated and Extruded Castor Meal Feeding in Laying Hens on the Internal Quality Parameters of Eggs

Parameter	Diet #, Substitution level (%), Week					
	0, 0, 1	0, 0, 2	0, 0, 3	0, 0, 4	0, 0, 5	0, 0, 6
EGGWT	59.53 (3.35)	57.09 (0.13)	59.29 (1.90)	59.50 (0.56)	57.07 (0.04)	60.23 (1.21)
YLKWT	16.25 (0.99)	15.28 (0.05)	16.24 (0.69)	16.05 (0.16)	15.76 (0.06)	16.33 (0.46)
YLKHT	17.38 (1.45)	18.75 (0.07)	19.30 (1.09)	17.85 (0.88)	18.00 (0.28)	18.50 (0.66)
ALBHT	5.65 (0.50)	7.38 (0.63)	6.70 (0.47)	7.43 (0.14)	6.30 (0.14)	6.70 (0.18)
COLOR	5.84 (0.42)	6.33 (0.03)	6.00 (0.04)	6.50 (0.47)	5,67 (0.37)	6.67 (0.73)
SHETK	38.34 (0.94)	37.67 (1.41)	38.34 (0.47)	38.33 (0.00)	38.84 (0.23)	39.50 (1.17)
ALBDI	77.59 (5.08)	54.93 (2.38)	62.08 (1.62)	59.85 (5.40)	61.41 (3.10)	43.80 (3.69)
YLKDI	17.64 (0.24)	15.79 (0.29)	16.60 (0.74)	16.31 (0.11)	16.12 (0.78)	9.26 (0.49)
HDPRO	0.81 (0.05)	0.80 (0.02)	0.86 (0.03)	0.92 (0.00)	0.83 (0.02)	0.81 (0.00)

(APPENDIX I continued.)

Parameter	Diet #, Substitution level (%), Week					
	1, 5, 1	1, 5, 2	1, 5, 3	1, 5, 4	1, 5, 5	1, 5, 6
EGGWT	60.87 (0.70)	58.19 (0.00)	57.33 (3.94)	59.84 (0.12)	58.01 (0.07)	59.95 (1.22)
YLKWT	16.34 (1.19)	15.52 (0.57)	15.71 (1.63)	16.17 (0.40)	15.69 (0.34)	15.85 (0.98)
YLKHT	17.04 (0.37)	18.41 (0.74)	18.35 (0.53)	18.39 (0.69)	16.75 (1.16)	18.54 (0.94)
ALBHT	6.27 (0.00)	7.75 (0.81)	6.37 (1.18)	6.90 (0.33)	5.78 (0.40)	7.10 (0.71)
COLOR	5.00 (0.26)	5.67 (0.32)	5.00 (0.91)	5.17 (0.40)	4.84 (0.31)	6.00 (0.62)
SHETK	39.68 (1.43)	39.00 (0.00)	38.34 (0.94)	38.50 (2.59)	39.34 (0.47)	39.00 (0.47)
ALBDI	67.60 (3.54)	56.83 (7.02)	62.19 (1.35)	62.84 (2.69)	62.29 (1.60)	42.79 (7.70)
YLKDI	17.36 (0.96)	16.24 (0.68)	16.19 (0.56)	16.99 (0.25)	16.07 (0.01)	8.55 (0.08)
HDPRO	0.82 (0.03)	0.81 (0.06)	0.81 (0.02)	0.86 (0.03)	0.88 (0.05)	0.84 (0.01)
	1, 10, 1	1, 10, 2	1, 10, 3	1, 10, 4	1, 10, 5	1, 10, 6
EGGWT	56.64 (1.67)	55.44 (1.27)	56.15 (1.67)	60.32 (0.56)	58.02 (2.28)	57.64 (2.34)
YLKWT	14.83 (0.29)	14.42 (0.52)	15.28 (0.16)	15.73 (0.49)	15.84 (0.36)	16.26 (1.51)
YLKHT	19.17 (0.09)	16.47 (0.47)	17.75 (0.68)	19.13 (0.36)	19.87 (0.57)	18.25 (0.40)
ALBHT	8.03 (0.28)	6.52 (0.07)	6.28 (0.07)	7.43 (1.17)	7.45 (0.21)	6.92 (0.12)
COLOR	5.00 (0.44)	4.67 (0.02)	4.17 (0.04)	3.67 (0.08)	3.67 (0.88)	5.50 (0.13)
SHETK	37.00 (0.47)	38.67 (0.47)	37.17 (0.23)	38.00 (0.47)	37.84 (0.23)	37.84 (0.23)
ALBDI	59.15 (4.50)	64.82 (4.49)	60.20 (2.89)	59.37 (5.02)	41.85 (6.32)	60.00 (0.77)
YLKDI	15.80 (0.28)	15.67 (0.62)	15.15 (0.91)	15.09 (0.10)	8.02 (0.15)	15.40 (0.62)
HDPRO	0.71 (0.11)	0.51 (0.18)	0.64 (0.20)	0.67 (0.11)	0.70 (0.14)	0.64 (0.01)

(APPENDIX I continued.)

Parameter	Diet #, Substitution level (%), Week					
	2, 5, 1	2, 5, 2	2, 5, 3	2, 5, 4	2, 5, 5	2, 5, 6
EGGWT	56.02 (1.73)	57.73 (2.38)	58.89 (0.30)	60.25 (2.96)	57.24 (0.86)	59.03 (0.35)
YLKWT	14.82 (0.51)	15.15 (0.45)	15.31 (0.09)	16.05 (0.63)	15.42 (0.28)	15.36 (0.24)
YLKHT	16.74 (0.19)	17.82 (0.83)	19.09 (0.13)	18.79 (0.30)	16.87 (0.05)	18.42 (0.16)
ALBHT	6.80 (0.24)	7.30 (0.28)	7.42 (0.40)	8.13 (0.14)	5.89 (0.45)	7.08 (0.21)
COLOR	4.67 (0.05)	4.67 (0.49)	4.50 (0.09)	5.17 (0.20)	5.36 (0.11)	5.67 (0.52)
SHETK	36.50 (1.65)	37.00 (0.47)	37.50 (0.71)	37.00 (0.95)	38.17 (0.23)	38.34 (0.94)
ALBDI	57.39 (2.62)	55.04 (5.16)	54.17 (0.71)	54.70 (3.18)	66.87 (0.98)	44.84 (0.03)
YLKDI	16.82 (1.33)	16.40 (0.11)	15.54 (0.03)	16.44 (0.06)	16.60 (0.06)	8.59 (0.13)
HDPRO	0.72 (0.21)	0.76 (0.16)	0.81 (0.14)	0.85 (0.13)	0.78 (0.07)	0.77 (0.16)
	2, 10, 1	2, 10, 2	2, 10, 3	2, 10, 4	2, 10, 5	2, 10, 6
EGGWT	60.29 (2.21)	57.93 (0.74)	57.94 (0.21)	59.27 (2.94)	58.17 (2.23)	60.17 (2.47)
YLKWT	15.42 (1.74)	15.43 (0.69)	15.00 (0.72)	15.95 (0.63)	16.48 (0.08)	17.86 (1.22)
YLKHT	17.99 (0.30)	17.22 (0.30)	19.14 (0.62)	17.66 (0.66)	19.34 (0.94)	18.87 (0.28)
ALBHT	6.74 (1.32)	6.85 (0.17)	7.33 (0.19)	7.35 (0.11)	6.53 (0.00)	7.40 (0.95)
COLOR	4.00 (0.39)	5.00 (0.25)	4.50 (0.04)	4.84 (0.28)	5.50 (0.04)	5.17 (0.06)
SHETK	39.50 (1.65)	39.33 (1.41)	37.83 (0.71)	38.67 (0.47)	39.00 (0.47)	37.17 (0.13)
ALBDI	69.01 (8.01)	64.49 (4.78)	55.57 (0.46)	59.34 (2.01)	43.82 (0.62)	57.39 (0.51)
YLKDI	16.51 (2.31)	16.48 (1.15)	15.57 (0.89)	16.64 (0.47)	9.93 (0.58)	16.07 (0.86)
HDPRO	0.89 (0.02)	0.82 (0.07)	0.94 (0.01)	0.91 (0.00)	0.91 (0.01)	0.90 (0.02)

(APPENDIX I continued.)

Parameter	Diet #, Substitution level (%), Week					
	3, 5, 1	3, 5, 2	3, 5, 3	3, 5, 4	3, 5, 5	3, 5, 6
EGGWT	59.88 (1.26)	62.36 (0.71)	59.11 (3.53)	61.48 (1.92)	58.46 (0.24)	60.84 (0.86)
YLKWT	15.49 (0.37)	17.25 (0.77)	16.44 (0.86)	16.57 (0.07)	15.36 (0.21)	16.84 (0.92)
YLKHT	18.33 (0.71)	18.83 (0.74)	18.21 (0.34)	19.08 (0.28)	18.45 (0.45)	18.39 (0.02)
ALBHT	6.52 (0.64)	6.99 (0.98)	6.80 (1.13)	7.94 (0.16)	7.05 (1.16)	6.07 (0.14)
COLOR	5.84 (0.35)	5.00 (0.48)	4.17 (0.23)	5.17 (0.25)	4.49 (0.14)	6.17 (0.35)
SHETK	38.50 (0.71)	38.34 (0.47)	37.84 (0.23)	38.00 (0.47)	37.67 (0.00)	37.17 (2.12)
ALBDI	62.32 (0.63)	61.31 (8.58)	60.43 (0.71)	55.96 (2.88)	58.84 (4.44)	44.20 (0.33)
YLKDI	15.97 (0.14)	16.79 (0.87)	17.56 (1.24)	16.30 (0.14)	14.79 (0.35)	8.67 (0.21)
HDPRO	0.81 (0.01)	0.91 (0.04)	0.88 (0.01)	0.90 (0.02)	0.91 (0.05)	0.86 (0.03)
	3, 10, 1	3, 10, 2	3, 10, 3	3, 10, 4	3, 10, 5	3, 10, 6
EGGWT	57.27 (0.50)	59.00 (0.91)	59.56 (0.33)	59.71 (2.33)	58.67 (0.28)	58.71 (0.46)
YLKWT	15.56 (0.49)	15.71 (0.18)	15.68 (0.59)	15.26 (0.62)	16.17 (0.63)	15.91 (0.23)
YLKHT	17.90 (0.10)	17.70 (0.10)	18.77 (0.76)	17.64 (0.09)	18.67 (0.05)	18.52 (0.30)
ALBHT	6.35 (0.17)	7.07 (0.66)	7.18 (0.81)	7.42 (0.40)	5.22 (0.54)	7.75 (0.35)
COLOR	4.17 (0.23)	5.00 (0.21)	5.50 (0.39)	4.17 (0.04)	4.50 (0.10)	5.17 (0.17)
SHETK	37.34 (0.47)	37.83 (0.71)	36.84 (1.18)	37.67 (0.00)	37.34 (0.47)	38.00 (0.47)
ALBDI	67.55 (1.45)	64.05 (3.22)	59.39 (5.26)	62.79 (2.61)	51.88 (2.31)	54.83 (1.61)
YLKDI	17.03 (1.61)	15.89 (0.14)	16.06 (0.20)	16.32 (0.05)	8.56 (0.62)	16.23 (1.35)
HDPRO	0.82 (0.07)	0.77 (0.02)	0.82 (0.09)	0.84 (0.05)	0.76 (0.08)	0.80 (0.16)

(APPENDIX I continued.)

Parameter	Diet #, Substitution level (%), Week					
	4, 5, 1	4, 5, 2	4, 5, 3	4, 5, 4	4, 5, 5	4, 5, 6
EGGWT	59.16 (6.01)	57.12 (2.63)	63.03 (3.33)	59.08 (2.48)	58.87 (0.36)	59.64 (0.61)
YLKWT	15.32 (0.25)	14.76 (0.58)	15.75 (0.53)	15.25 (0.37)	15.75 (0.80)	16.15 (0.09)
YLKHT	18.02 (0.92)	17.32 (0.26)	18.08 (1.63)	17.77 (0.66)	17.80 (0.38)	18.24 (0.49)
ALBHT	6.02 (0.73)	7.05 (0.31)	6.44 (2.50)	7.27 (0.80)	6.65 (0.88)	6.38 (0.76)
COLOR	4.67 (0.37)	5.00 (0.16)	6.34 (0.69)	5.84 (0.19)	5.34 (0.19)	6.17 (2.36)
SHETK	38.17 (1.18)	37.67 (1.89)	37.00 (0.47)	38.17 (0.23)	40.00 (0.47)	38.00 (5.42)
ALBDI	66.24 (1.02)	57.35 (1.28)	66.88 (4.07)	61.52 (4.00)	60.07 (0.42)	44.79 (0.56)
YLKDI	16.01 (0.27)	15.38 (0.02)	16.01 (0.42)	15.44 (0.22)	16.19 (0.27)	8.31 (0.02)
HDPRO	0.88 (0.05)	0.86 (0.06)	0.89 (0.01)	0.84 (0.08)	0.93 (0.04)	0.91 (0.02)
	4, 10, 1	4, 10, 2	4, 10, 3	4, 10, 4	4, 10, 5	4, 10, 6
EGGWT	60.08 (1.58)	59.94 (2.49)	62.87 (3.18)	63.00 (0.23)	59.07 (1.24)	62.93 (2.15)
YLKWT	15.38 (0.21)	15.46 (0.08)	16.03 (1.00)	16.54 (0.47)	15.82 (0.31)	16.38 (0.76)
YLKHT	17.20 (0.57)	17.40 (0.38)	18.97 (0.14)	19.22 (0.16)	19.45 (0.07)	18.30 (0.38)
ALBHT	6.42 (0.26)	6.74 (0.76)	7.15 (0.11)	7.95 (0.31)	6.42 (0.59)	7.20 (0.39)
COLOR	4.00 (0.00)	4.84 (0.16)	5.17 (0.05)	5.33 (0.00)	5.50 (1.01)	5.33 (0.08)
SHETK	37.17 (1.18)	39.67 (1.41)	37.17 (0.71)	38.00 (0.47)	38.50 (0.24)	38.00 (0.95)
ALBDI	71.71 (0.65)	65.01 (1.82)	62.95 (1.66)	61.20 (2.47)	46.50 (8.10)	60.95 (0.91)
YLKDI	16.55 (0.60)	15.54 (0.31)	16.48 (0.17)	16.05 (0.48)	10.06 (0.07)	16.32 (0.85)
HDPRO	0.79 (0.03)	0.76 (0.09)	0.84 (0.07)	0.91 (0.01)	0.94 (0.02)	0.82 (0.09)

(APPENDIX I continued.)

Parameter	Diet #, Substitution level (%), Week					
	5, 5, 1	5, 5, 2	5, 5, 3	5, 5, 4	5, 5, 5	5, 5, 6
EGGWT	62.64 (7.01)	58.72 (0.06)	58.72 (1.29)	60.13 (0.60)	59.81 (1.63)	61.56 (3.41)
YLKWT	15.29 (0.01)	15.77 (1.03)	16.42 (0.31)	15.30 (0.20)	16.30 (0.25)	16.37 (1.37)
YLKHT	16.97 (0.47)	16.75 (0.17)	18.28 (0.18)	17.48 (0.35)	18.02 (0.07)	17.80 (0.33)
ALBHT	5.49 (0.45)	5.80 (0.89)	6.50 (0.66)	6.98 (0.01)	6.17 (0.23)	6.18 (0.35)
COLOR	5.84 (0.19)	6.00 (0.04)	5.50 (0.43)	5.50 (0.31)	5.50 (0.23)	5.83 (0.25)
SHETK	36.34 (1.89)	37.70 (1.65)	38.83 (0.71)	38.34 (0.47)	38.50 (0.24)	39.50 (1.17)
ALBDI	70.14 (2.10)	70.26 (8.88)	62.03 (4.71)	67.02 (2.80)	64.83 (0.38)	45.79 (0.01)
YLKDI	16.48 (0.86)	17.30 (0.59)	16.79 (0.14)	16.11 (0.40)	16.66 (0.78)	9.25 (0.06)
HDPRO	0.86 (0.02)	0.92 (0.03)	0.95 (0.01)	0.94 (0.00)	0.93 (0.00)	0.96 (0.02)
	5, 10, 1	5, 10, 2	5, 10, 3	5, 10, 4	5, 10, 5	5, 10, 6
EGGWT	61.78 (0.18)	59.81 (0.88)	60.67 (2.09)	61.74 (0.45)	60.34 (1.98)	63.16 (0.57)
YLKWT	15.74 (0.35)	16.03 (0.82)	16.35 (1.51)	17.18 (0.13)	16.87 (0.27)	16.61 (0.89)
YLKHT	16.75 (0.11)	17.79 (0 02)	19.32 (0.78)	18.02 (0.07)	19.19 (0.27)	18.30 (0.24)
ALBHT	5.34 (0.37)	6.97 (0.80)	6.85 (0.25)	6.77 (0.18)	6.07 (0.90)	7.45 (0.74)
COLOR	3.84 (0.16)	5.00 (0.14)	5.17 (0.21)	5.34 (0.25)	5.17 (0.57)	5.00 (0.02)
SHETK	38.67 (1.89)	36.67 (0.94)	37.00 (1.41)	38.50 (0.71)	38.84 (0.23)	39.17 (1.65)
ALBDI	76.29 (3.68)	63.22 (1.95)	61.62 (1.44)	63.86 (3.23)	49.98 (7.49)	59.14 (3.33)
YLKDI	17.22 (1.65)	16.14 (0.16)	16.50 (1.44)	16.86 (0.33)	9.43 (0.79)	16.64 (1.41)
HDPRO	0.84 (0.06)	0.84 (0.06)	0.89 (0.03)	0.81 (0.04)	0.90 (0.06)	0.82 (0.11)

[a] 0, control (soy bean meal) treatment; 1, Untreated castor meal; 2, 2% sodium hydroxide treatment; 3, 2% sodium bicarbonate treatment; 4, 2% calcium hydroxide treatment; and 5, 1% sodium hydroxide

(APPENDIX I continued.)

+ 1% sodium hypochlorite treatment.

[b] EGGWT, egg weight (g); YLKWT, yolk weight (g); YLKHT, yolk height (g); ALBHT, albumin height (mm); COLOR, La-Rouch color fan value; SHETK, shell thickness (mm x 100); ALBDI, albumin diameter (mm); YLKDI, yolk diameter (mm x ½); and HDPRO, hen day production (%).

[c] Each value is the mean (standard deviation) of 20 determinations.

APPENDIX II

Results of Duncan's Multiple Comparison Test[a] on Internal Egg Quality Parameters[b] of the Laying Hens Study in the Aspects of Chemically Treated and Extruded Diets[c] and the Level of their Substitution Ratio in the Soy Bean Meal

Parameter	Mean comparasion based on																
	Diet #											Level, %				
[Week 1]																	
EGGWT	(6)		(5)		(1)		(2)		(4)		(3)	(5)		(10)		(0)
	59.5		58.3		57.0		56.5		56.3		53.5	57.2		56.6		56.5
	A	a	A	a	A	a	A	a	A	a	A	A	a	A	a	A
YLKWT	(1)		(2)		(4)		(6)		(5)		(3)	(0)		(5)		(10)
	16.3		15.6		15.5		15.5		15.4		15.1	16.23		15.5		15.4
	A	a	A	a	A	a	A	a	A	a	A	A	a	A	a	A
YLKHT	(4)		(2)		(5)		(1)		(3)		(6)	(0)		(5)		(10)
	18.1		18.1		17.6		17.4		17.4		16.9	17.8		17.4		17.4
	A	a	A	a	A	a	A	a	A			A	a	A	a	A
					B	b	B	b	B	b	B					
ALBHT	(2)		(3)		(4)		(5)		(1)		(6)	(10)		(5)		(0)
	7.2		6.8		6.4		6.2		5.7		5.4	6.6		6.2		5.7
	A	a	A	a	A	a	A					A	a	A		
					B	b	B	b	B					B	b	B
							C	c	C	c	C					
COLOR	(1)		(4)		(2)		(6)		(5)		(3)	(0)		(5)		(10)
	5.8		5.0		5.0		4.8		4.3		1.3	5.8		5.2		4.2
	A											A				
			B	b	B	b	B							B		
							C	c	C	c	C					C
SHETK	(2)		(1)		(3)		(4)		(5)		(6)	(0)		(10)		(5)
	38.3		38.3		38.0		37.9		37.7		37.5	38.3		37.9		37.8
	A	a	A	a	A	a	A	a	A	a	A	A	a	A	a	A
ALBDI	(1)		(6)		(5)		(4)		(2)		(3)	(0)		(10)		(5)
	77.6		73.2		69.0		64.9		63.4		62.2	77.6		68.3		64.7
	A	a	A	a	A							A				
			B	b	B	b	B	b	B					B	b	B
					C	c	C	c	C	c	C					
YLKDI	(1)		(6)		(3)		(2)		(4)		(5)	(0)		(10)		(5)
	17.6		16.9		16.7		16.6		16.5		16.3	17.6		16.6		16.0
	A	a	A	a	A	a	A	a	A	a	A	A	a	A	a	A
HDPRO	(6)		(5)		(4)		(1)		(3)		(2)	(5)		(10)		(0)
	0.85		0.83		0.81		0.81		0.80		0.76	0.82		0.81		0.81
	A	a	A	a	A	a	A	a	A	a	A	A	a	A	a	A

(APPEXDIX II continued.)

Parameter	Mean comparasion based on								
	Diet #						Level,%		
[Week 2]									
EGGWT	(4)	(6)	(5)	(3)	(1)	(2)	(5)	(10)	(0)
	60.7	59.3	58.5	57.8	57.1	56.8	58.8	58.4	57.1
	A a	A a	A				A a	A a	A
		B b	B b	B b	B b	B			
YLKWT	(4)	(6)	(3)	(1)	(5)	(2)	(5)	(10)	(0)
	16.5	15.9	15.3	15.3	15.1	15.0	15.7	15.4	15.3
	A a	A					A a	A a	A
		B b	B b	B b	B b	B			
YLKHT	(1)	(4)	(3)	(2)	(5)	(6)	(0)	(5)	(10)
	18.8	18.3	17.5	17.4	17.4	17.3	18.8	17.8	17.3
	A a	A					A		
		B b	B					B b	B
			C c	C c	C c	C			
ALBHT	(1)	(2)	(3)	(4)	(5)	(6)	(0)	(5)	(10)
	7.4	7.1	7.1	7.0	6.9	6.4	7.4	7.0	6.8
	A a	A a	A a	A a	A a	A	A a	A a	A
COLOR	(1)	(6)	(2)	(4)	(5)	(3)	(0)	(5)	(10)
	6.3	5.5	5.2	5.0	4.9	4.8	6.3	5.3	4.9
	A						A		
		B b	B					B b	B
			C c	C c	C c	C			
SHETK	(2)	(5)	(3)	(4)	(1)	(6)	(10)	(5)	(0)
	38.8	38.7	38.2	38.1	37.7	37.0	38.4	37.8	37.7
	A a	A a	A a	A a	A a	A	A a	A a	A
ALBDI	(6)	(4)	(5)	(2)	(3)	(1)	(10)	(5)	(0)
	66.7	62.7	61.2	60.8	59.8	54.9	64.3	60.1	54.9
	A a	A a	A a	A a	A		A a	A	
		B b	B b	B b	B b	B		B b	B
YLKDI	(6)	(3)	(4)	(2)	(1)	(5)	(5)	(10)	(0)
	16.7	16.4	16.3	16.0	15.8	15.5	16.4	16.0	15.3
	A a	A a	A a	A a	A		A a	A a	A
		B b	B b	B b	B b	B			
HDPRO	(6)	(4)	(5)	(1)	(3)	(2)	(5)	(0)	(10)
	0.88	0.84	0.81	0.80	0.79	0.66	0.85	0.80	0.74
	A a	A a	A a	A a	A		A a	A a	A
			B b	B b	B b	B			

(APPEXDIX II continued.)

Parameter	Mean comparasion based on	
	Diet #	Level,%
[Week 3]		
EGGWT	(5) (6) (4) (1) (3) (2) 63.0 60.0 59.3 59.3 58.9 56.7 A a A a A a A a A b b b b b b b b b	(5) (0) (10) 59.6 59.4 59.3 A a A a A
YLKWT	(6) (1) (4) (5) (2) (3) 16.4 16.2 16.1 15.9 15.5 15.2 A a A a A a A a A a A	(0) (5) (10) 16.2 15.9 15.7 A a A a A
YLKHT	(1) (3) (6) (5) (4) (2) 19.3 19.1 18.8 18.5 18.5 18.1 A a A a A a A a A a A	(0) (10) (5) 19.3 18.8 18.4 A a A a A
ALBHT	(3) (4) (5) (1) (6) (2) 7.4 7.0 6.8 6.7 6.7 6.3 A a A a A a A a A a A	(10) (5) (0) 7.0 6.7 6.7 A a A a A
COLOR	(1) (5) (6) (4) (2) (3) 6.0 5.8 5.3 4.8 4.6 4.5 A a A a A B b B b B b B	(0) (5) (10) 6.0 5.1 4.9 A B b B
SHETK	(1) (6) (2) (3) (4) (5) 38.3 38.0 32.8 37.7 37.3 37.1 A a A a A a A a A a A	(0) (5) (10) 38.3 38.0 37.2 A a A a A
ALBDI	(5) (1) (6) (2) (4) (3) 64.9 62.1 61.8 61.2 59.9 54.9 A a A a A a A a A a A	(0) (5) (10) 62.1 61.1 60.0 A a A a A
YLKDI	(4) (6) (1) (5) (2) (3) 16.8 16.7 16.6 16.3 15.7 15.6 B b B b B b B b B b B	(0) (5) (10) 16.6 16.5 16.0 B b B b B
HDPRO	(6) (3) (1) (5) (4) (2) 0.92 0.88 0.86 0.86 0.85 0.73 A a A a A a A a A B b B b B b B b B	(5) (0) (10) 0.87 0.86 0.83 A a A a A B b B b B

(APPEXDIX II continued.)

Parameter	Mean comparasion based on								
	Diet #						Level,%		
[Week 4]									
EGGWT	(5)	(6)	(4)	(2)	(3)	(1)	(10)	(5)	(0)
	61.0	60.9	60.6	60.1	59.8	59.5	60.8	60.2	59.5
	A a	A a	A a	A a	A a	A	A a	A a	A
YLKWT	(6)	(1)	(3)	(2)	(4)	(5)	(10)	(5)	(0)
	16.2	16.1	16.0	16.0	16.0	15.9	16.1	16.1	15.9
	A a	A a	A a	A a	A a	A	A a	A a	A
YLKHT	(2)	(5)	(4)	(3)	(1)	(6)	(10)	(5)	(0)
	18.8	18.5	18.4	18.2	17.9	17.3	18.3	18.3	17.9
	A a	A a	A a	A			A a	A a	A
		B b	B b	B b	B b	B	B b	B b	B
ALBHT	(3)	(4)	(5)	(1)	(2)	(6)	(5)	(10)	(0)
	7.7	7.7	7.6	7.4	7.2	6.9	7.4	7.4	7.4
	A a	A a	A a	A a	A a	A	A a	A a	A
COLOR	(1)	(5)	(6)	(3)	(4)	(2)	(0)	(5)	(10)
	6.5	5.6	5.4	5.0	4.7	4.4	6.5	5.4	4.7
	A						A		
		B b	B b	B b	B			B b	B
				C c	C c	C			
SHETK	(6)	(1)	(2)	(5)	(4)	(3)	(0)	(10)	(5)
	38.4	38.3	38.3	38.1	37.8	37.8	38.3	38.2	35.0
	A a	A a	A a	A a	A a	A	A a	A a	A
ALBDI	(6)	(5)	(2)	(1)	(4)	(3)	(10)	(5)	(0)
	65.4	61.4	61.1	59.9	59.4	57.0	61.3	60.4	60.0
	A a	A a	A a	A a	A		A a	A a	A
		B b	B b	B b	B b	B			
YLKDI	(3)	(6)	(1)	(4)	(2)	(5)	(0)	(5)	(10)
	16.5	16.5	16.3	16.3	16.0	15.8	16.3	16.3	16.2
	A a	A a	A a	A a	A		A a	A a	A
					B b	B			
HDPRO	(1)	(3)	(6)	(5)	(4)	(2)	(0)	(5)	(10)
	0.92	0.88	0.88	0.88	0.87	0.76	0.92	0.88	0.83
	A a	A a	A a	A a	A		A a	A a	A
						B	B b	B b	B

(APPENDIX II continued.)

Parameter	Mean comparasion based on								
	Diet #						Level,%		
[Week 5]									
EGGWT	(6)	(5)	(4)	(2)	(3)	(1)	(10)	(5)	(0)
	61.1	59.3	58.6	58.0	57.7	57.1	59.0	58.5	57.1
	A a	A a	A a	A a	A		A a	A a	A
		B b	B b	B b	B b	B			
YLKWT	(6)	(3)	(5)	(2)	(4)	(1)	(10)	(0)	(5)
	16.6	16.0	15.8	15.8	15.8	15.8	16.2	15.8	15.7
	A						A a	A a	A
		B b	B b	B b	B b	B			
YLKHT	(5)	(6)	(4)	(2)	(3)	(1)	(10)	(0)	(5)
	18.6	18.6	18.6	18.3	18.1	18.0	19.3	18.0	17.6
	A a	A a	A a	A a	A a	A	A		
								B b	B
ALBHT	(2)	(5)	(1)	(3)	(4)	(6)	(10)	(5)	(0)
	6.6	6.5	6.3	6.2	6.1	6.1	6.3	6.3	6.3
	A a	A a	A a	A a	A a	A	A a	A a	A
COLOR	(1)	(5)	(3)	(6)	(4)	(2)	(0)	(5)	(10)
	5.7	5.4	5.4	5.3	4.5	4.3	5.7	5.1	4.9
	A a	A a	A a	A			A a	A a	A
					B b	B			
		C c	C c	C c	C				
SHETK	(5)	(1)	(6)	(2)	(3)	(4)	(0)	(5)	(10)
	39.3	38.8	38.7	38.6	38.6	37.5	38.8	38.7	38.3
	A a	A a	A				A a	A	
		B b	B b	B b	B			B b	B
						C			
ALBDI	(1)	(6)	(4)	(3)	(5)	(2)	(5)	(0)	(10)
	61.4	57.4	55.4	55.3	53.3	52.1	62.6	61.4	46.8
	A a	A a	A a	A			A a	A	
		B b	B b	B b	B b	B			B
YLKDI	(1)	(3)	(5)	(6)	(2)	(4)	(0)	(5)	(10)
	1615	13.3	13.1	13.1	12.0	11.7	16.1	16.1	9.2
	A						A a	A	
		B b	B b	B					B
					C c	C			
HDPRO	(5)	(6)	(3)	(4)	(1)	(2)	(5)	(10)	(0)
	0.94	0.91	0.84	0.83	0.83	0.79	0.88	0.84	0.83
	A a	A a	A a	A a	A		A a	A a	A
			B b	B b	B b	B	B b	B b	B

(APPENDIX II continued.)

Parameter	Mean comparasion based on								
	Diet #						Level,%		
[WeeK 6]									
EGGWT	(6)	(5)	(1)	(4)	(3)	(2)	(10)	(0)	(5)
	62.4	61.3	60.2	59.8	59.6	58.8	60.5	60.2	60.2
	A a	A a	A a	A a	A		A a	A a	A
		B b	B b	B b	B b	B			
YLKWT	(3)	(6)	(4)	(1)	(5)	(2)	(10)	(0)	(5)
	16.6	16.5	16.4	16.3	16.2	16.1	16.6	16.3	16.1
	A a	A a	A a	A a	A a	A	A a	A a	A
YLKHT	(3)	(1)	(4)	(2)	(5)	(6)	(10)	(0)	(5)
	18.6	18.5	18.5	18.4	18.3	18.1	18.5	18.5	18.3
	A a	A a	A a	A a	A a	A	A a	A a	A
ALBHT	(3)	(2)	(4)	(6)	(5)	(1)	(10)	(0)	(5)
	7.2	6.8	6.4	6.2	5.7	5.4	6.6	6.2	5.7
	A a	A a	A a	A a	A a	A	A a	A	
								B b	B
COLOR	(1)	(2)	(5)	(4)	(3)	(6)	(0)	(5)	(10)
	6.7	5.8	5.8	5.7	5.4	5.4	6.7	6.0	5.2
	A a	A a	A a	A			A a	A	
		B b	B b	B b	B b	B		B b	B
SHETK	(1)	(6)	(2)	(5)	(3)	(4)	(0)	(10)	(5)
	38.3	38.3	38.0	37.9	37.7	37.5	38.3	37.9	37.8
	A a	A a	A a	A a	A a	A	A a	A a	A
ALBDI	(5)	(6)	(2)	(3)	(4)	(1)	(10)	(5)	(0)
	52.9	52.5	51.4	51.1	49.5	43.8	58.5	46.5	43.8
	A a	A a	A a	A a	A		A		
						B		B b	B
YLKDI	(6)	(4)	(3)	(5)	(2)	(1)	(10)	(0)	(5)
	12.9	12.5	12.3	12.3	12.0	9.3	16.1	9.3	8.7
	A a	A a	A a	A a	A		A		
						B		B b	B
HDPRO	(6)	(5)	(1)	(3)	(4)	(2)	(5)	(0)	(10)
	0.89	0.87	0.86	0.84	0.83	0.74	0.87	0.81	0.80
	A a	A a	A a	A a	A		A a	A a	A
		B b	B b	B b	B b	B			

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[a] Numbers of the same letters are not significantly different at

(APPENDIX II continued.)

$P < 0.1$.

[b] EGGWT, egg weight (g); YLKWT, yolk weight (g); YLKHT, yolk height (mm); ALBHT, albumin height (mm); COLOR, La-Rouche color fan value; SHETK, shell thickness(x100) (mm); ALBDI, albumin diameter (mm); YLKDI, yolk diameter (mm/2); and HDPRO, hen day production of egg.

[c] Diet 0, soy bean meal (Control); Diet 1, untreated castor meal; Diet 2, 2% sodium hydroxide treatment; Diet 3, 2% sodium bicarbonate treatment; Diet 4, 2% caicium hydroxide treatment; and Diet 5, 1% sodium hydroxide + 1% sodium hypochlorite treatment.

APPENDIX III

Incidence of Histological Observations[a] in Swine Fed with Chemically Treated and Extruded Castor Meal

	Diet #					
	0	1	2	3	4	5
Esophogus						
No significant lesions						
Stomach						
1. Hyperemia	40	20	10	-	33	20
2. Lymphocytic accumulations in the submucosa	-	-	-	-	22	-
3. Eosinophil infiltration in the submucosa, mild	-	-	-	10	11	-
4. Eosinophil and lymphocytic infiltration between smooth muscle layers	-	-	-	-	11	-
5. Eosinophil infiltration into the lamina propria	-	-	20	-	-	10
6. Lymphocytic infiltration into the lamina propria	10	-	-	-	11	-
7. Lymphocytic infiltration into the muscularis	-	-	20	-	-	-
8. Fibrous thickening of stroma in the lamina propria	-	-	-	-	-	10
Duodenum						
1. Plasma cell infiltration into the lamina propria						
a. Minimal	-	-	-	20	-	-
b. Mild	70	70	90	60	55	80
c. Moderate	20	30	-	20	44	10
2. Eosinophil infiltration into the lamina propria						
a. Minimal	-	-	-	-	11	10
b. Moderate	-	-	-	-	-	-
Jejunum						
1. Eosinophil infiltration into the lamina propria						
a. Minimal	40	40	60	50	22	20

(APPENDIX III continued)

	Diet #					
	0	1	2	3	4	5
b. Mild	50	50	20	40	55	60
c. Moderate	-	-	10	-	22	10
d. Heavy	10	-	-	-	-	-
2. Plasma cell infiltration into the lamina propria						
a. Minimal	20	-	10	20	-	10
b. Mild	-	20	-	-	-	-
c. Moderate	10	-	10	-	11	-
3. Lymphocyte infiltration into the lamina propria						
a. Mild	-	10	-	-	-	10
b. Moderate	10	-	-	-	-	-
Ileum						
1. Eosinophil infiltration into the lamina propria						
a. Minimal	50	20	60	40	22	50
b. Mild	30	50	10	30	55	40
c. Moderate	-	10	-	-	-	-
2. Plasma cell infiltration into the lamina propria						
a. Minimal	10	-	-	-	-	-
b. Mild	-	-	-	10	-	20
c. Moderate	-	10	-	-	11	-
3. Neutrophil infiltration (focal) into the lamina propria	-	10	-	-	-	-
4. Neutrophil in intestinal crypts	10	20	-	-	-	-
5. Hyperemia	-	20	-	-	-	-
6. Lymphocyte and histocyte infiltration into the lamina propria, heavy	10	10	-	-	-	-
7. Histocyte infiltration into the lamina propria,						

(APPENDIX III continued.)

	Diet #					
	0	1	2	3	4	5
moderate	10	-	-	-	-	-
Cecum						
1. Plasma cell infiltration into the lamina propria						
a. Minimal	60	40	80	80	55	60
b. Mild	40	40	10	10	22	20
c. Moderate	-	-	-	-	22	20
2. Eosinophil infiltration into the lamina propria						
a. Minimal	-	10	-	-	-	20
b. Moderate	-	-	-	-	-	10
Spiral colon						
1. Plasma cell infiltration into the lamina propria						
a. Minimal	50	60	60	40	77	50
b. Mild	50	50	20	30	11	30
c. Moderate	-	-	-	-	11	-
Descending colon						
1. Plasma cell infiltration into the lamina propria						
a. Minimal	60	20	50	40	22	50
b. Mild	20	40	10	10	44	30
c. Moderate	10	-	-	-	22	-
Liver						
1. Lymphocytic foci. randomly distributed	-	20	30	10	-	20
2. Eosinophil infiltration, portal tracts	-	10	-	-	-	10
3. Focal necrosis, lumphocyte infiltration	-	10	-	-	-	20

(APPENDIX III continued.)

	Diet #					
	0	1	2	3	4	5
Pancreas						
No significant lesions						
Lung						
1. Hemorrhage	20	50	40	40	11	20
2. Parabronchlolar lymphocyte nodules	40	40	30	60	44	40
3. Emphysema	60	40	30	40	22	40
4. Neutrophil infiltration, mild	-	-	10	10	-	20
5. Atelectasis	-	-	-	20	11	20
6. Mucopurulent bronchitis	-	-	10	20	-	10
Heart						
1. Focal necrosis, infiltration of eosinophils and lymphocytes	-	10	-	-	-	-
2. Focal subepicardial accumulation of lymphocytes, histocytes and neutrophils	10	-	-	-	-	-
Kidney						
1. Focal accumulations of lymphocytes						
a. Cortex	20	20	-	20	22	-
b. Medulla	20	20	40	20	11	-
2. Cystic	-	10	-	-	-	-
Urinary bladder						
1. Submucosal lymphocyte foci	-	-	-	10	-	-
2. Submucosal focus of plasma cells	-	10	-	10	-	-

(APPENDIX III continued.)

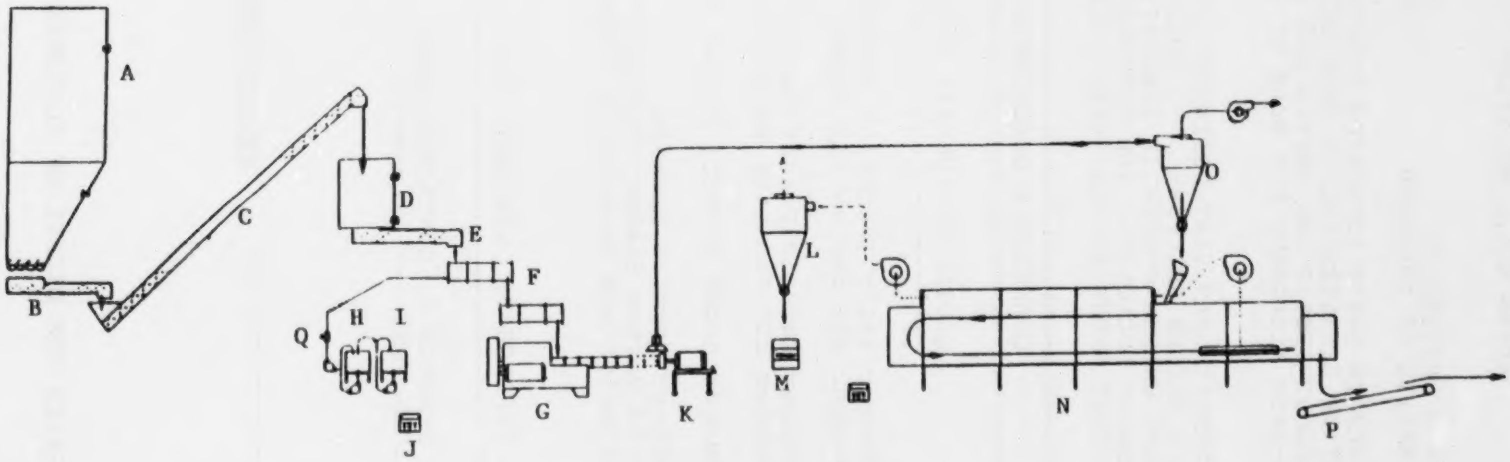
	Diet #					
	0	1	2	3	4	5
Spleen						
1. Cytoplasmic vacuolation of R.E. cells	10	-	-	-	-	10
2. Lymphocyte hyperplasia, eosinophil infiltration, mild	-	-	-	10	-	-
3. R.E. hyperplasia	-	-	10	-	-	-
Mesenteric Lymph Node						
1. Lymphocyte hyperplasia	40	-	20	10	11	20
2. Lymphocyte depletion	-	-	-	-	-	30
3. Hematoma, cyst formation	-	-	-	-	-	10

[a] Values are percentages based on 10 pigs per treatment except values for treatment Diet 5 which are based on 9 pigs

[b] 0, soy bean meal (control); 1, untreated castor meal; 2, 2% sodium hydroxide treatment; 3, 2% sodium bicarbonate treatment; 4, 2% calcium hydroxide treatment; and 5, 1% sodium hydroxide + 1% sodium hypochlorite treatment.

APPENDIX IV

Proposed Equipment Layout, Specifications, and Cost Estimates



A	Raw material bin	J	Control panel
B	Live bottom feeder	K	Knife drive
C	Screw conveyor	L	Dryer exhaust collector
D	Circular bin	M	Trash collector
E	Feed screw	N	Dryer/cooler
F	Double conditioning cylinder	O	Negative pneumatic collector
G	Extruder	P	Belt conveyor
H	Feed tank	Q	Rotameter
I	Mix tank		

WENGER INTERNATIONAL, INC.
KANSAS CITY, MO.,U.S.A. - ANTWERPEN, BELGIUM
TITLE:
TEXAS A&M - WENGER
PROCESS FLOW DIAGRAM FOR
CASTOR BEAN DETOXIFICATION

[illegible]	DRAWN BY: D. BALDWIN	SCALE: NONE	DRAWING NO. 2357 REV. A
	APP. BY: G.L.J.	DATE: MAR 14, 1986	SH. 1 OF 1

WENGER OVERSEAS, INC.
One Crown Center, Suite 510
2400 Pershing Rd.
Kansas City, MO 64108 U.S.A.

X-175/DETOXIFICATION

FIRM: TEXAS A&M UNIVERSITY
FOR: BRASIL OR THAILAND

DATE: April 4, 1986
SPECIAL Q1E 1-315-84

PROPOSAL FOR WENGER X-175 EXTRUSION COOKING SYSTEM FOR DETOXIFICATION OF DEFATTED CASTOR BEAN MEAL AS PER TRIALS IN WENGER PILOT PLANT 850717 AND 850826 AND FLOW DIAGRAM NO. 29671 AT 2550-2800 KILOGRAMS PER HOUR OF FINAL PRODUCT AT 8% MOISTURE.

CAPACITY AND DENSITY CAN VARY DEPENDING ON RAW MATERIALS USED. ELECTRIC MOTORS ARE FOR VOLT CYCLE AND ARE CONSTANT RPM UNLESS OTHERWISE SPECIFIED. ALL BELT GUARDS ARE TOTALLY ENCLOSED. ALL COMPONENTS IN CARBON STEEL EXCEPT WHERE SHOWN AS STAINLESS STEEL.

QUOTATION SUMMARY SHEET

ITEM	QUANTITY & DESCRIPTION	PRICE
1.	One each Wenger X-175 Extrusion Cooker.	390,611.00
2.	One each Wenger 600 Series III Dryer/Cooler.	137,232.00
3.	One each Wenger Caustic Feeder Package.	35,420.00
4.	One each Wenger 12' Sanitary Belt Conveyor.	8,116.00
5.	One each Wenger Motor Control Center with Starters.	36,507.00
6.	One each Wenger Service Package.	25,200.00
7.	One each Live Bottom Feeder, Conveyor Screw, Collector and Negative Air System.	68,543.00

TOTAL, ITEMS 1 THROUGH 7, UNPACKED, FOB SABETHA, KANSAS ------------------------------ $ 701,629.00

TOTAL, ITEMS 1 THROUGH 7, CONTAINERIZED, FAS NORTH AMERICAN PORT ---------------------- $ 707,154.00

ACCEPTED BY: ____________________

____________________ WENGER OVERSEAS, INC.

SEE FOLLOWING PAGES FOR DETAIL OF EQUIPMENT, TERMS AND WARRANTY.

WENGER

ITEM QUANTITY & DESCRIPTION PRICE

1. One each Wenger X-175 continuous extrusion cooker (right hand model) including all of the following:
   - Extruder components for eight extruder sections with required heads, screws, steamlocks, and final die. Screws and steamlocks all mounted on splined drive shaft.
   - All extruder section components where available that come in contact with the product are of alloy steel construction.
   - Steam injectors and manifold as needed for extruder barrel.
   - Main drive assembly to receive eight head extruder section. V-belt drive with sheaves, belts, and 200 HP, 1500 RPM drive motor.
   - Mild steel circular bin discharger 48" (1219mm) OD x 72" (1829mm), 75 cubic ft. (2.1 cu. m.) capacity for use as a live bottom bin to uniformity feed extruder preconditioner or feeder screw, complete with:
     - 5 HP constant RPM motor and drive with gear reducer.
     - Switches for signaling high and low levels of raw material.
     - Inspection door, coned bridge breaker, cover with manhole, 4" (102mm) vent opening and two sight glasses.
     - Manual operated cable hoist to lift cone for cleaning.
   - Stainless steel 6" x 84" (152mm x 2134mm) feeder screw with clean out door for feeding raw ingredients from circular bin discharger to mixing cylinder. 2 HP varispeed drive motor.
   - Two each all stainless steel double shaft conditioning cylinder 92" x 20" (2337mm x 508mm) diameter with gear reducer and 15 HP V-belt drive and motor, SS shaft and beaters, steam manifold, liquid inlets, SS downspout with thermometer in centigrade and by-pass valve.
   - General temperature control system and instrumentation package for above extruder including the following:
     - Necessary valves and manifold for manually controlling the flow of water or steam through the head jackets of the different extruder section temperature zones in order to help maintain desired cooking temperatures.
     - Hoses for connection from head jacket valve manifold to head jackets.

CONTINUED

  - Prewired control panel with dust tight hinged door. Door with lexan see-through insert.
  - Stop/start push-buttons and lights with one key operated push-button lockout for safety purposes.
  - Hourmeter.
  - Ammeter for main motor.
  - Single zone temperature indicator.
  - Ammeter coil for main drive motor, maximum 600V.
  - Thermocouples and lead wire assembly.
  - Steam pressure control accessory kit for extruder head jackets.
  - Water pressure control accessory kit for water injection into product.
  - Water rotameter for indicating quantity of water injected into product in inlet head.
  - Water rotameter for indicating quantity of water injected into product in conditioning cylinder.
  - Steam pressure control accessory kit for product steam injection in conditioning cylinder.
  - Steam pressure control accessory kit for product steam injection in extruder barrel.
  - Tachometer for feeder motor.

One each Wenger external drive knife assembly including:

- Base assembly, frame, casters with locks and drive shaft.
- One each stainless steel hood over knife.
- Motor starting lockout device for customer's padlock.
- 5 HP varispeed drive motor.
- One each Wenger ball bearing knife assembly with holder and blades for use with X-155 final dies.

TOTAL FOR ITEM 1 ----------------------- 390,611.00

2. One each Wenger Series III Model 600, two stage perforated tray dryer/cooler. Modular design with housing built in seven foot (2130mm) sections. Upper stage driven by a 1 HP, varispeed motor and lower stage driven by 1 HP, varispeed motor. Unit includes:

- Upper stage with two sections of dryer and lower stage with two sections of dryer and two attached 7 foot single stage cooler zones.

CONTINUED

- Housing in the form of insulated panels with fiber glass insulation. Inside and outside sheets aluminized steel. All side panels where possible will be doors which can be opened for cleaning the inside of the unit. Housing built in 7 foot (2130mm) sections and designed for containerization.
- Support frame furnished (maximum 5 feet, 1500mm).
- Galvanized steel perforated product carrying trays.
- Circulating fans for each dryer section driven by 7.5 HP motor(s). Dryer exhaust fan with 10 HP motor plus separate cooler fan with 10 HP motor.
- Fines recovery system wiping the bottom plate of dryer/cooler. Fines are deposited into fines screw conveyors which carry the fines to the side of the dryer/cooler. Two fines screws furnished on this unit driven by two 3/4 HP gear motors.
- Oscillating product spreader driven by air cylinder.
- Steam coils constructed in accordance with A.S.M.E. standards for steam at 10 kg per square cm. Automatic temperature regulator, indicating controller and traps furnished.
- Prewired electrical control panel of dust tight construction with push-buttons and pilot lights for each motor.
- Perforated trays not installed in unit so that the internal body of the unit can be filled with other items during shipment in order to minimize freight. Customer must connect steam coil manifolds or gas burners and mount controls. Wenger to furnish prints and instructions for this installation.

TOTAL FOR ITEM 2 ----------------------- 137,232.00

3. One each Wenger caustic solution feed package, to include:
   - Specifications:
     - Sized to feed caustic solution at 20% of dry ingredient feed rate.
     - Caustic solution to consist of 5% NaOH, 5% NaOCL, and 90% HOH.
   - Mix tank:
     - Steam jacketed.
     - 200 gallon (760 liter) working capacity.
     - Sealed construction with threaded ports

CONTINUED

for introduction of ingredients.

  - Safety type overflow vent connection.
  - Guarded liquid level sight glass to be field calibrated for measuring ingredients.
  - Centrifugal type recirculation and transfer pump with 3/4 HP, constant speed motor.
  - Three-way manual ball valve to divert recirculated solution to fill feed tank.
  - Additional required plumbing devices, shipped loose for customer mounting, including pressure gauge, thermometer, steam controller, steam pressure relief valve, and steamtrap (customer to furnish shut-off valves and piping).
- Feed tank:
  - Steam jacketed.
  - 200 gallon (760 liter) working capacity.
  - Sealed construction with inlet piped to mix tank three-way valve.
  - Safety type overflow vent connection.
  - Guarded liquid level sight glass.
  - Additional required plumbing devices, shipped loose for customer mounting, including pressure gauge, thermometer, steam controller, steam pressure relief valve, and steamtrap (customer to furnish shut-off valves and piping).
- Feed pump:
  - Peristaltic type metering pump, mounted to feed tank, belt driven, with 3/4 HP variable speed motor.
  - Norphene type tubing with quantity for approximately 10,000 hours of operation.
  - Suitable safety shields.
- Flow meter:
  - Armored rotameter type, suitable for caustic solutions.
  - Calibrated for 1% accuracy.
- Control panel:
  - Additional start/stop push-buttons and pilot lights for each motor, mounted in extruder control panel.
  - Customer to furnish wiring as required between control panel, motor control center and other devices.
- Materials:
  - All metallic material contacting solution to be 304 or 316 stainless

CONTINUED

steel.
  - All non-metallic materials contacting solution to be of a caustic resistant type.
- Installation:
  - Customer to install tanks as close as possible to conditioning cylinder.
  - Customer to furnish means of introducing ingredients into mix tank.
  - Customer to mount rotameter and furnish piping between pump and rotameter, and between rotameter and conditioning cylinder.

TOTAL FOR ITEM 3 ------------------------ 35,420.00

4. One each Wenger 12 foot (3660mm) sanitary inclined belt conveyor with chain drive, motor base, 3/4 HP gear motor. Frame of rigid aluminum construction with replaceable stainless steel liner on all wear surfaces and contact points. Collects product from cooler discharge. Also includes:
- 14 inch (355mm) wide high temperature white belting, with cleats.
- Stainless steel inlet hopper.
- Support bracket for mounting belt at discharge of cooler.

TOTAL FOR ITEM 4 ------------------------ 8,116.00

5. One each motor control enclosure, 28 space factors, 400 amp:
- Enclosure:
  - Free standing with legs.
  - NEMA 12 (dust tight), mild steel construction.
  - Two doors with key lock handle.
  - 72 inch (1825mm) high, by 60 inch (1525mm) wide, by 10 inch 250mm) deep.
- Main circuit breaker:
  - 600 volt, 3 pole, 400 amp maximum.
  - External disconnect handle.
- Includes 16 each NEMA Size 1 starters, 4 each NEMA Size 2 starters, and leach NEMA Size 3 starter.
- Customer will be required to furnish 3 phase power and single phase control power to each enclosure and to wire between enclosure and motors and control panels.

One each motor control enclosure, reduced voltage starting, NEMA Size 5:

CONTINUED

- Enclosure:
  - Wall mounted.
  - NEMA 12 (dust tight), mild steel construction.
  - 70.25 inch (1785mm) high, by 33.25 inch (845mm) wide by 16.00 inch (405mm) deep.
  - Single door.
- Circuit breaker:
  - 600 volt, 3 pole, 366 amp maximum.
  - Handle operated through door.
- Includes contractors for reduced voltage starting and thermal overload relays.
- Prewired to a numbered terminal strip.
- Lockout push-button located on exterior of enclosure door.
- Customer will be required to furnish three phase power and single phase control power to enclosure and to wire between enclosure and motor and control panel.
- 150 HP maximum at 230 V and 250 HP maximum at 380-575 V.

TOTAL FOR ITEM 5 ------------------------ 36,507.00

6. Services area consisting of the following:
   - Three sets of instruction manuals in English language including installation instruction, parts diagrams, parts lists, electrical diagrams, plumbing diagrams and dimension prints for each assembly.

WW170100 - Detailed layout of equipment suitable for client's center-line equipment placement.

WW170200 - Start-up commissioning services of Wenger delegated specialist(s) for a period of up to 14 man days of absence from hometown. Client to pay for all transportation, lodging, meals and out-of-pocket expenses while specialist away from hometown.

- The necessary visa (if any required) are to be procured by Wenger but client will arrange for the necessary local paperwork if any required.
- In case of the need of extended stay of our specialist(s) the following rates are agreed:
  - For each further day: U.S. $400.00 per day of absence from hometown, valid until December 31, 1986.

CONTINUED

ITEM	QUANTITY & DESCRIPTION	PRICE
WW170300	- Training services in U.S.A. to train/educate the delagation of end user (up to three men for one continuous week). - Such training/education comprises a general technical introduction in the construction/operation of the machinery, assembling of the single parts, maintenance, operation, safety-measures, etc. - The costs for all air travel necessary, meals, lodging, etc., for this training to be paid by client except that of Wenger personnel. Wenger to provide access to its pilot plant for three (3) days during this training and to pay for raw materials up to a value of up to U.S. $1,000.00.	
WW170400 through WW170499	- Spare parts and tools for perpetual inventory, general maintenance and repair of equipment. Detailed and itemized parts list is available after receipt of order. Non-Wenger spares to be obtained directly from vendors (rather than from Wenger) following this initial supply.	
	TOTAL FOR ITEM 6 ------------------------	25,200.00

"The following equipments are not manufactured by Wenger, and therefore, carry the individual suppliers mechanical guarantees. The overall suitability of the equipment for the function stated is backed by Wenger. Future operating spare parts for these equipments should be ordered directly from the individual suppliers".

7. WN020500	- One live bottom feeder, with (6) 9" diameter variable pitch screw feeder 8'-0" long, complete with a 3 HP variable speed drive, ERC and drive components. Feeder troughs will be fabricated from 10-gauge carbon steel. - One transition, fabricated from 10-gauge carbon steel, between the discharge of the live bottom feeder and the 6" diameter inclined screw conveyor. The transition will be	

CONTINUED

approximately 6'-0" wide with a 30 degree inclined slope on one end.

- One 6" diameter tube type screw conveyor 40'-0" long. The 30 degree inclined conveyor will have a 10-gauge tube and be complete with a 3 HP motor with gear reducer and drive components.
- Four rotary level indicators, paddle type, two for the customer's raw material holding bin and two for the Wenger extruder circular bin.
- One dryer/cooler collector fabricated from 10 and 12-gauge carbon steel. The positive type collector will be complete with an inlet transition and exhaust stack with manual damper. Includes airlock with 1/2 HP motor.
- One each negative airlift conveying system designed to convey cooked product from extruder to dryer.
- Included in this system are the following:
  - One each straight bladed fan Class 5 construction, Arr. 9 with standard stainless steel wheel and carbon steel housing. Features included are:
    - V-belt drive and drive guard.
    - Adjustable motor base on side of fan.
    - 1-1/4" N.P.T. drain.
    - Outlet damper opposed blade, 25 HP motor.
  - One each flat top cyclone receiver of 12 gauge stainless steel construction with 10" flanged product discharge. Other features include:
    - 6" O.D. inlet stub.
    - 8" O.D. draw through exhaust fitting.
    - 8" bolted door in cone.
  - One each heavy duty cast rotary airlock 12 x 10 complete with 8-vane stainless steel rotor, double lip shaft seals, outboard bearings. Also included are:
    - Hard chrome bore.
    - 1 HP drive assembly including: motor, right angle gear reducer, roller chain drive, drive guard, and motor base completely factory assembled.
  - One each take-away hopper of stainless steel construction.

CONTINUED

ITEM	QUANTITY & DESCRIPTION	PRICE
	- One each group of vacuum conveying line.	
	TOTAL FOR ITEM 7 ------------------------	68,543.00

TOTAL, ITEMS 1 THROUGH 7, UNPACKED, FOB SABETHA, KANSAS -------------------------------- $ 701,629.00

TERMS: 20% down payment with order plus irrevocable letter of credit (confirmed by acceptable U.S.A. bank) to be opened within 30 days for balance of order. Letter of credit to be valid for 180 days from opening date and to be available against 1) our commercial invoice; 2) packing list; and 3) draft at sight against the letter of credit, plus in the event of CIF purchase; 4) accepted for carriage or received for shipment ocean bill of lading (in the case of container shipment customer's letter of credit MUST indicate container shipments permissable); and 5) all risk warehouse to warehouse marine insurance certificate for a minimum of 110% of the CIF value. Shipment from North America port with number of days specified in above terms for letter of credit validity. This delivery time is initiated after receipt of your down payment and formal written purchase order in our Kansas City office.

It will expedite handling procedures if the U.S.A. bank which confirms the letter of credit can then advise letter of credit through UNITED MISSOURI BANK OF KANSAS CITY, MISSOURI, N.A. who are our bankers. Their proximity permits us to present bills of lading, etc., promptly after receipt, thus helping you to have documents in your hands in a timely manner as soon as possible after shipment occurs.

NOTE: Please note that equipment manufactured by Wenger is covered by Warranty as per attached sheet. This Warranty is an integral part of this proposal and voids any other condition put forth by purchaser.

NOT INCLUDED IN THE FOREGOING PROPOSAL BUT NECESSARY FOR THE OPERATION OF THE SYSTEM, ARE THESE GENERAL PURPOSE ITEMS WHICH SHOULD BE SECURED FROM OTHERS, OR MAY ALREADY BE AVAILABLE IN YOUR PLANT.

CONTINUED

ITEM QUANTITY & DESCRIPTION PRICE

1 set Ducting from dryer exhaust fan to atmosphere or to customer's collector.

1 ea. Boiler to produce 10 kilograms per square centimeter steam to service extruder and dryer.

1 set Raw material mixing equipment and product conveying equipment as required by plant layout.

1 set Ancillary equipment which is not included in this quotation but which is required for customer's installation.

NOTE: The equipment as included in this quotation is fabricated and/or assembled in our manufacturing plant and then is disassembled only enough to permit the most economical shipment. Prior to shipment of the equipment Wenger will send an instruction manual with details concerning the final utility connections which the customer must make in his plant. After the equipment has been installed in your plant, Wenger has available a service technician who can come to your plant to start the equipment, train your operators and discuss maintenance of the equipment with your maintenance personnel.

CONTINUED

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 8 MAXIMUM CAPACITY (KG/HR): 2800

CAPACITY:

This production rate has been determined from averages of several formulations. Variations can occur due to several contingencies such as raw ingredients, moisture, final product characteristics, and shape. The capacity shown is before application of external flavor and before drying unless a moisture content out of the dryer is shown.

CONSUMPTION:

The "normal usage" figures indicate what the machine will consume during normal operating conditions.

The "sizing info" figures are to be used for sizing the utility services to the equipment. These consumptions may occur during some operating situations.

CONSUMPTION/kg OF PRODUCT PRODUCED:

These figures are shown for comparison to other machine sizes. The "TOTAL" refers to the consumption per hour and the "consumption/kg of product produced" is shown on the line beneath it.

STEAM:

Although the equipment will operate at lower steam pressures we recommend 9-10 kg/cm2. If steam is used for the dryer it can be returned to the boiler. Steam for jacketed heads cannot be returned.

WATER:

Constant water pressure of 2-4 kg/cm2 must be available. For injection into the product during cooking we recommend that this be at a temperature of approximately 65-90 C. Cooling water for the jacketed heads of the machine should be approximately 4-32 C and could rise in temperature 8-10 C maximum after circulation through the jackets.

COMPRESSED AIR:

A pressure of 1.5-2 kg/cm2 is needed for operation of controls. This air must be clean, dry, and oil free.

GAS:

Our consumption figures are based on natural gas at 9000 kcal/Nm3. If you plan to use a gas dryer please omit the steam consumption shown for the dryer. If a steam heated dryer will be used omit the gas requirement.

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 8 MAXIMUM CAPACITY (KG/HR): 2800

ELECTRICAL CONSUMPTION (kW)

(EXTRUDER)

LOCATION	NORMAL USAGE	SIZING INFO
main drive	119.4	149.2
mixing cylinder	9.0	11.2
feeder screw	1.2	1.5
circular bin	3.0	3.7
knife drive	3.0	3.7
extra cond.cyl.	9.0	11.2
live bin feeder	1.8	2.2
screw conveyor	1.8	2.2
TOTAL	148.0	185.0
kWh/kg PRODUCED	0.053	

ELECTRICAL CONSUMPTION (kW)

(DRYER AND/OR COOLER)

LOCATION	NORMAL USAGE	SIZING INFO
main conveyor(s)	1.2	1.5
fines auger(s)	0.9	1.1
circulating fan(s)	9.0	11.2
exhaust fan	6.0	7.5
cooler fan	6.0	7.5
recirc.pump	0.4	0.6
feed pump	0.4	0.6

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 8 MAXIMUM CAPACITY (KG/HR): 2800

ELECTRICAL CONSUMPTION (kW)

(DRYER AND/OR COOLER)

LOCATION	NORMAL USAGE	SIZING INFO
belt conveyor	0.4	0.6
airlocks	0.9	1.1
pneumatic fan	14.9	18.7
TOTAL	40.1	50.2
kWh/kg PRODUCED	0.014	

STEAM CONSUMPTION (kg/h)

LOCATION	NORMAL USAGE	SIZING INFO
into product	168.0	223.9
extruder jackets	7.4	29.5
dryer	952.0	1269.3
feed tank	25.0	40.0
TOTAL	1152.4	1562.7
kg/kg PRODUCED	0.412	

WATER CONSUMPTION (l/h)

LOCATION	NORMAL USAGE	SIZING INFO
into product	280.0	560.0
extruder jackets	1392.0	1856.0
TOTAL	1672.0	2416.0
l/kg PRODUCED	0.597	

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 8 MAXIMUM CAPACITY (KG/HR): 2800

AIR CONSUMPTION (Nl/h)

LOCATION	NORMAL USAGE	SIZING INFO
dryer/cooler	920.4	1840.8
product spreader	30.0	60.0
TOTAL	950.4	1900.8
Nl/kg PRODUCED	0.339	

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          WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG                                              REV: A
04/03/86      DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175                                          DENSITY (G/L): 400
NUMBER OF HEADS: 8                    MAXIMUM CAPACITY (KG/HR): 2800
====================================================================

  THESE UTILITY CONSUMPTION FIGURES WERE CALCULATED BASED ON THE
  FOLLOWING ASSUMPTIONS:

                 % STEAM INTO PRODUCT: 6.0
                 2 EXTRUDER JACKETS USING STEAM
                 % MOISTURE OUT OF EXTRUDER: 25.0
                 % MOISTURE OF PRODUCT INTO DRYER: 25
                 % MOISTURE OF PRODUCT OUT OF DRYER: 8
                 % WATER INTO PRODUCT: 10
                 0 STEAM FLOW METERS
                 0 PRESSURIZED PANELS

                 SYSTEM UTILITY COST PER TON ANALYSIS

                 EXTRUDER ELECTRICAL          $    3.70
                 DRYER/COOLER ELECTRICAL      $    1.00
                 STEAM                        $    8.23
                 WATER                        $    0.30
                 AIR                          $     .00
                 TOTAL COST PER TON           $   13.24

                 SYSTEM ENERGY COST ASSUMPTIONS

                 $    0.070 PER KILOWATT
                 $   20.000 PER MT STEAM
                 $    0.500 PER CUBIC METER WATER
                 $    0.150 PER CUBIC METER GAS
                 $    0.014 PER CUBIC METER AIR
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WENGER OVERSEAS, INC.
One Crown Center, Suite 510
2400 Pershing Rd.
Kansas City, MO 64108 U.S.A.

X-175/DETOXIFICATION

FIRM: TEXAS A&M UNIVERSITY
FOR: BRASIL OR THAILAND

DATE: April 4, 1986
SPECIAL Q1E 1-316-84

PROPOSAL FOR WENGER X-175 EXTRUSION COOKING SYSTEM FOR DETOXIFICATION OF DEFATTED CASTOR BEAN MEAL AS PER TRIALS IN WENGER PILOT PLANT 850717 AND 850826 AND FLOW DIAGRAM NO. 29671 AT 1100-1400 KILOGRAMS PER HOUR OF FINAL PRODUCT AT 8% MOISTURE.

CAPACITY AND DENSITY CAN VARY DEPENDING ON RAW MATERIALS USED. ELECTRIC MOTORS ARE FOR VOLT CYCLE AND ARE CONSTANT RPM UNLESS OTHERWISE SPECIFIED. ALL BELT GUARDS ARE TOTALLY ENCLOSED. ALL COMPONENTS IN CARBON STEEL EXCEPT WHERE SHOWN AS STAINLESS STEEL.

QUOTATION SUMMARY SHEET

ITEM	QUANTITY & DESCRIPTION	PRICE
1.	One each Wenger X-175 Extrusion Cooker.	289,391.00
2.	One each Wenger 600 Series III Dryer/Cooler.	104,324.00
3.	One each Wenger Caustic Feeder Package.	30,844.00
4.	One each Wenger 12' Sanitary Belt Conveyor.	8,116.00
5.	One each Wenger Motor Control Center with Starters.	35,543.00
6.	One each Wenger Service Package.	22,700.00
7.	One each Live Bottom Feeder, Conveyor Screw, Collector and Negative Air System.	62,135.00

TOTAL, ITEMS 1 THROUGH 7, UNPACKED, FOB SABETHA, KANSAS ------------------------------ $ 553,053.00

TOTAL, ITEMS 1 THROUGH 7, CONTAINERIZED, FAS NORTH AMERICAN PORT ---------------------- $ 558,253.00

ACCEPTED BY: ______________________

______________________ WENGER OVERSEAS, INC.

SEE FOLLOWING PAGES FOR DETAIL OF EQUIPMENT, TERMS AND WARRANTY.

ITEM | QUANTITY & DESCRIPTION | PRICE

1. One each Wenger X-175 continuous extrusion cooker (right hand model) including all of the following:
   - Extruder components for six extruder sections with required heads, screws, steamlocks, and final die. Screws and steamlocks all mounted on splined drive shaft.
   - All extruder section components where available that come in contact with the product are of alloy steel construction.
   - Steam injectors and manifold as needed for extruder barrel.
   - Main drive assembly to receive six head extruder section. V-belt drive with sheaves, belts, and 150 HP, 1500 RPM drive motor.
   - Mild steel circular bin discharger 48" (1219mm) OD x 72" (1829mm), 75 cubic ft. (2.1 cu. m.) capacity for use as a live bottom bin to uniformity feed extruder preconditioner or feeder screw, complete with:
     - 5 HP constant RPM motor and drive with gear reducer.
     - Switches for signaling high and low levels of raw material.
     - Inspection door, coned bridge breaker, cover with manhole, 4" (102mm) vent opening and two sight glasses.
     - Manual operated cable hoist to lift cone for cleaning.
   - Stainless steel 6" x 84" (152mm x 2134mm) feeder screw with clean out door for feeding raw ingredients from circular bin discharger to mixing cylinder. 2 HP varispeed drive motor.
   - All stainless steel double shaft conditioning cylinder 92" x 20" (2337mm x 508mm) diameter with gear reducer and 15 HP V-belt drive and motor, SS shaft and beaters, steam manifold, liquid inlets, SS downspout with thermometer in centigrade and by-pass valve.
   - General temperature control system and instrumentation package for above extruder including the following:
     - Necessary valves and manifold for manually controlling the flow of water or steam through the head jackets of the different extruder section temperature zones in order to help maintain desired cooking temperatures.
     - Hoses for connection from head jacket valve manifold to head jackets.

CONTINUED

  - Prewired control panel with dust tight hinged door. Door with lexan see-through insert.
  - Stop/start push-buttons and lights with one key operated push-button lockout for safety purposes.
  - Hourmeter.
  - Ammeter for main motor.
  - Single zone temperature indicator.
  - Ammeter coil for main drive motor, maximum 600V.
  - Thermocouples and lead wire assembly.
  - Steam pressure control accessory kit for extruder head jackets.
  - Water pressure control accessory kit for water injection into product.
  - Water rotameter for indicating quantity of water injected into product in inlet head.
  - Water rotameter for indicating quantity of water injected into product in conditioning cylinder.
  - Steam pressure control accessory kit for product steam injection in conditioning cylinder.
  - Steam pressure control accessory kit for product steam injection in extruder barrel.
  - Tachometer for feeder motor.

One each Wenger external drive knife assembly including:

- Base assembly, frame, casters with locks and drive shaft.
- One each stainless steel hood over knife.
- Motor starting lockout device for customer's padlock.
- 5 HP varispeed drive motor.
- One each Wenger ball bearing knife assembly with holder and blades for use with X-155 final dies.

TOTAL FOR ITEM 1 ----------------------- 289,391.00

2. One each Wenger Series III Model 600, two stage perforated tray dryer/cooler. Modular design with housing built in seven foot (2130mm) sections. Upper stage driven by a 1 HP, varispeed motor and lower stage driven by 1 HP, varispeed motor. Unit includes:

- Upper stage with one section of dryer and lower stage with one section of dryer and one attached 7 foot single stage cooler zone.

CONTINUED

WENGER

- Housing in the form of insulated panels with fiber glass insulation. Inside and outside sheets aluminized steel. All side panels where possible will be doors which can be opened for cleaning the inside of the unit. Housing built in 7 foot (2130mm) sections and designed for containerization.
- Support frame furnished (maximum 5 feet, 1500mm).
- Galvanized steel perforated product carrying trays.
- Circulating fans for each dryer section driven by 7.5 HP motor(s). Dryer exhaust fan with 7.5 HP motor plus separate cooler fan with 7.5 HP motor.
- Fines recovery system wiping the bottom plate of dryer/cooler. Fines are deposited into fines screw conveyors which carry the fines to the side of the dryer/cooler. Two fines screws furnished on this unit driven by two 3/4 HP gear motors.
- Oscillating product spreader driven by air cylinder.
- Steam coils constructed in accordance with A.S.M.E. standards for steam at 10 kg per square cm. Automatic temperature regulator, indicating controller and traps furnished.
- Prewired electrical control panel of dust tight construction with push-buttons and pilot lights for each motor.
- Perforated trays not installed in unit so that the internal body of the unit can be filled with other items during shipment in order to minimize freight. Customer must connect steam coil manifolds or gas burners and mount controls. Wenger to furnish prints and instructions for this installation.

TOTAL FOR ITEM 2 ------------------------ 104,324.00

3. One each Wenger caustic solution feed package, to include:
   - Specifications:
     - Sized to feed caustic solution at 20% of dry ingredient feed rate.
     - Caustic solution to consist of 5% NaOH, 5% NaOCL, and 90% HOH.
   - Mix tank:
     - Steam jacketed.
     - 90 gallon (340 liter) working capacity.
     - Sealed construction with threaded ports

CONTINUED

  for introduction of ingredients.
  - Safety type overflow vent connection.
  - Guarded liquid level sight glass to be field calibrated for measuring ingredients.
  - Centrifugal type recirculation and transfer pump with 3/4 HP, constant speed motor.
  - Three-way manual ball valve to divert recirculated solution to fill feed tank.
  - Additional required plumbing devices, shipped loose for customer mounting, including pressure gauge, thermometer, steam controller, steam pressure relief valve, and steamtrap (customer to furnish shut-off valves and piping).
- Feed tank:
  - Steam jacketed.
  - 90 gallon (340 liter) working capacity.
  - Sealed construction with inlet piped to mix tank three-way valve.
  - Safety type overflow vent connection.
  - Guarded liquid level sight glass.
  - Additional required plumbing devices, shipped loose for customer mounting, including pressure gauge, thermometer, steam controller, steam pressure relief valve, and steamtrap (customer to furnish shut-off valves and piping).
- Feed pump:
  - Peristaltic type metering pump, mounted to feed tank, belt driven, with 3/4 HP variable speed motor.
  - Norphene type tubing with quantity for approximately 10,000 hours of operation.
  - Suitable safety shields.
- Flow meter:
  - Armored rotameter type, suitable for caustic solutions.
  - Calibrated for 1% accuracy.
- Control panel:
  - Additional start/stop push-buttons and pilot lights for each motor, mounted in extruder control panel.
  - Customer to furnish wiring as required between control panel, motor control center and other devices.
- Materials:
  - All metallic material contacting solution to be 304 or 316 stainless

CONTINUED

steel.
  - All non-metallic materials contacting solution to be of a caustic resistant type.
- Installation:
  - Customer to install tanks as close as possible to conditioning cylinder.
  - Customer to furnish means of introducing ingredients into mix tank.
  - Customer to mount rotameter and furnish piping between pump and rotameter, and between rotameter and conditioning cylinder.

TOTAL FOR ITEM 3 ------------------------ 30,844.00

4. One each Wenger 12 foot (3660mm) sanitary inclined belt conveyor with chain drive, motor base, 3/4 HP gear motor. Frame of rigid aluminum construction with replaceable stainless steel liner on all wear surfaces and contact points. Collects product from cooler discharge. Also includes:
- 14 inch (355mm) wide high temperature white belting, with cleats.
- Stainless steel inlet hopper.
- Support bracket for mounting belt at discharge of cooler.

TOTAL FOR ITEM 4 ------------------------ 8,116.00

5. One each motor control enclosure, 28 space factors, 400 amp:
- Enclosure:
  - Free standing with legs.
  - NEMA 12 (dust tight), mild steel construction.
  - Two doors with key lock handle.
  - 72 inch (1825mm) high, by 60 inch (1525mm) wide, by 10 inch (250mm) deep.
- Main circuit breaker:
  - 600 volt, 3 pole, 400 amp maximum.
  - External disconnect handle.
- Includes 16 each NEMA Size 1 starters and 4 each NEMA Size 2 starters.
- Customer will be required to furnish 3 phase power and single phase control power to each enclosure and to wire between enclosure and motors and control panels.

One each motor control enclosure, reduced voltage starting, NEMA Size 5:
- Enclosure:

CONTINUED

ITEM	QUANTITY & DESCRIPTION	PRICE

- Wall mounted.
- NEMA 12 (dust tight), mild steel construction.
- 70.25 inch (1785mm) high, by 33.25 inch (845mm) wide by 16.00 inch (405mm) deep.
- Single door.

- Circuit breaker:
  - 600 volt, 3 pole, 366 amp maximum.
  - Handle operated through door.
- Includes contractors for reduced voltage starting and thermal overload relays.
- Prewired to a numbered terminal strip.
- Lockout push-button located on exterior of enclosure door.
- Customer will be required to furnish three phase power and single phase control power to enclosure and to wire between enclosure and motor and control panel.
- 150 HP maximum at 230 V and 250 HP maximum at 380-575 V.

TOTAL FOR ITEM 5 ------------------------ 35,543.00

6. Services area consisting of the following:

- Three sets of instruction manuals in English language including installation instruction, parts diagrams, parts lists, electrical diagrams, plumbing diagrams and dimension prints for each assembly.

WW170100 - Detailed layout of equipment suitable for client's center-line equipment placement.

WW170200 - Start-up commissioning services of Wenger delegated specialist(s) for a period of up to 14 man days of absence from hometown. Client to pay for all transportation, lodging, meals and out-of-pocket expenses while specialist away from hometown.

- The necessary visa (if any required) are to be procured by Wenger but client will arrange for the necessary local paperwork if any required.
- In case of the need of extended stay of our specialist(s) the following rates are agreed:
  - For each further day: U.S. $400.00 per day of absence from hometown, valid until December 31, 1986.

WW170300 - Training services in U.S.A. to train/

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educate the delagation of end user (up to three men for one continuous week).

- Such training/education comprises a general technical introduction in the construction/operation of the machinery, assembling of the single parts, maintenance, operation, safety-measures, etc.
- The costs for all air travel necessary, meals, lodging, etc., for this training to be paid by client except that of Wenger personnel. Wenger to provide access to its pilot plant for three (3) days during this training and to pay for raw materials up to a value of up to U.S. $1,000.00.

WW170400 through WW170499 - Spare parts and tools for perpetual inventory, general maintenance and repair of equipment. Detailed and itemized parts list is available after receipt of order. Non-Wenger spares to be obtained directly from vendors (rather than from Wenger) following this initial supply.

TOTAL FOR ITEM 6 ---------------------- 22,700.00

"The following equipments are not manufactured by Wenger, and therefore, carry the individual suppliers mechanical guarantees. The overall suitability of the equipment for the function stated is backed by Wenger. Future operating spare parts for these equipments should be ordered directly from the individual suppliers".

7.

WN020500 - One live bottom feeder, with (6) 9" diameter variable pitch screw feeder 8'-0" long, complete with a 3 HP variable speed drive, ERC and drive components. Feeder troughs will be fabricated from 10-gauge carbon steel.

- One transition, fabricated from 10-gauge carbon steel, between the discharge of the live bottom feeder and the 6" diameter inclined screw conveyor. The transition will be approximately 6'-0" wide with a 30

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degree inclined slope on one end.

- One 6" diameter tube type screw conveyor 40'-0" long. The 30 degree inclined conveyor will have a 10-gauge tube and be complete with a 3 HP motor with gear reducer and drive components.
- Four rotary level indicators, paddle type, two for the customer's raw material holding bin and two for the Wenger extruder circular bin.
- One dryer/cooler collector fabricated from 10 and 12-gauge carbon steel. The positive type collector will be complete with an inlet transition and exhaust stack with manual damper. Includes airlock with 1/2 HP motor.
- One each negative airlift conveying system designed to convey cooked product from extruder to dryer.
- Included in this system are the following:
  - One each straight bladed fan Class 5 construction, Arr. 9 with standard stainless steel wheel and carbon steel housing. Features included are:
    - V-belt drive and drive guard.
    - Adjustable motor base on side of fan.
    - 1-1/4" N.P.T. drain.
    - Outlet damper opposed blade, 15 HP motor.
  - One each flat top cyclone receiver of 12 gauge stainless steel construction with 10" flanged product discharge. Other features include:
    - 6" O.D. inlet stub.
    - 8" O.D. draw through exhaust fitting.
    - 8" bolted door in cone.
  - One each heavy duty cast rotary airlock 12 x 10 complete with 8-vane stainless steel rotor, double lip shaft seals, outboard bearings. Also included are:
    - Hard chrome bore.
    - 1 HP drive assembly including: motor, right angle gear reducer, roller chain drive, drive guard, and motor base completely factory assembled.
  - One each take-away hopper of stainless steel construction.
  - One each group of vacuum conveying

CONTINUED

line.

TOTAL FOR ITEM 7 ------------------------ 62,135.00

TOTAL, ITEMS 1 THROUGH 7, UNPACKED, FOB SABETHA, KANSAS -------------------------------- $ 553,053.00

TERMS: 20% down payment with order plus irrevocable letter of credit (confirmed by acceptable U.S.A. bank) to be opened within 30 days for balance of order. Letter of credit to be valid for 180 days from opening date and to be available against 1) our commercial invoice; 2) packing list; and 3) draft at sight against the letter of credit, plus in the event of CIF purchase; 4) accepted for carriage or received for shipment ocean bill of lading (in the case of container shipment customer's letter of credit MUST indicate container shipments permissable); and 5) all risk warehouse to warehouse marine insurance certificate for a minimum of 110% of the CIF value. Shipment from North America port with number of days specified in above terms for letter of credit validity. This delivery time is initiated after receipt of your down payment and formal written purchase order in our Kansas City office.

It will expedite handling procedures if the U.S.A. bank which confirms the letter of credit can then advise letter of credit through UNITED MISSOURI BANK OF KANSAS CITY, MISSOURI, N.A. who are our bankers. Their proximity permits us to present bills of lading, etc., promptly after receipt, thus helping you to have documents in your hands in a timely manner as soon as possible after shipment occurs.

NOTE: Please note that equipment manufactured by Wenger is covered by Warranty as per attached sheet. This Warranty is an integral part of this proposal and voids any other condition put forth by purchaser.

NOT INCLUDED IN THE FOREGOING PROPOSAL BUT NECESSARY FOR THE OPERATION OF THE SYSTEM, ARE THESE GENERAL PURPOSE ITEMS WHICH SHOULD BE SECURED FROM OTHERS, OR MAY ALREADY BE AVAILABLE IN YOUR PLANT.

1 set Ducting from dryer exhaust fan to atmosphere or

CONTINUED

to customer's collector.

1 ea. Boiler to produce 10 kilograms per square centimeter steam to service extruder and dryer.

1 set Raw material mixing equipment and product conveying equipment as required by plant layout.

1 set Ancillary equipment which is not included in this quotation but which is required for customer's installation.

NOTE: The equipment as included in this quotation is fabricated and/or assembled in our manufacturing plant and then is disassembled only enough to permit the most economical shipment. Prior to shipment of the equipment Wenger will send an instruction manual with details concerning the final utility connections which the customer must make in his plant. After the equipment has been installed in your plant, Wenger has available a service technician who can come to your plant to start the equipment, train your operators and discuss maintenance of the equipment with your maintenance personnel.

CONTINUED

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 6 MAXIMUM CAPACITY (KG/HR): 1400

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CAPACITY:

This production rate has been determined from averages of several formulations. Variations can occur due to several contingencies such as raw ingredients, moisture, final product characteristics, and shape. The capacity shown is before application of external flavor and before drying unless a moisture content out of the dryer is shown.

CONSUMPTION:

The "normal usage" figures indicate what the machine will consume during normal operating conditions.

The "sizing info" figures are to be used for sizing the utility services to the equipment. These consumptions may occur during some operating situations.

CONSUMPTION/kg OF PRODUCT PRODUCED:

These figures are shown for comparison to other machine sizes. The "TOTAL" refers to the consumption per hour and the "consumption/kg of product produced" is shown on the line beneath it.

STEAM:

Although the equipment will operate at lower steam pressures we recommend 9-10 kg/cm2. If steam is used for the dryer it can be returned to the boiler. Steam for jacketed heads cannot be returned.

WATER:

Constant water pressure of 2-4 kg/cm2 must be available. For injection into the product during cooking we recommend that this be at a temperature of approximately 65-90 C. Cooling water for the jacketed heads of the machine should be approximately 4-32 C and could rise in temperature 8-10 C maximum after circulation through the jackets.

COMPRESSED AIR:

A pressure of 1.5-2 kg/cm2 is needed for operation of controls. This air must be clean, dry, and oil free.

GAS:

Our consumption figures are based on natural gas at 9000 kcal/Nm3. If you plan to use a gas dryer please omit the steam consumption shown for the dryer. If a steam heated dryer will be used omit the gas requirement.

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 6 MAXIMUM CAPACITY (KG/HR): 1400

ELECTRICAL CONSUMPTION (kW)

(EXTRUDER)

LOCATION	NORMAL USAGE	SIZING INFO
main drive	89.5	111.9
mixing cylinder	9.0	11.2
feeder screw	1.2	1.5
circular bin	3.0	3.7
knife drive	3.0	3.7
live bin feeder	1.8	2.2
screw conveyor	1.8	2.2
TOTAL	109.2	136.5
kWh/kg PRODUCED	0.078	

ELECTRICAL CONSUMPTION (kW)

(DRYER AND/OR COOLER)

LOCATION	NORMAL USAGE	SIZING INFO
main conveyor(s)	1.2	1.5
fines auger(s)	0.9	1.1
circulating fan(s)	4.5	5.6
exhaust fan	4.5	5.6
cooler fan	4.5	5.6
recirc.pump	0.4	0.6
feed pump	0.4	0.6
belt conveyor	0.4	0.6

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 6 MAXIMUM CAPACITY (KG/HR): 1400

ELECTRICAL CONSUMPTION (kW)

(DRYER AND/OR COOLER)

LOCATION	NORMAL USAGE	SIZING INFO
airlocks	0.9	1.1
pneumatics fan	9.0	11.2
TOTAL	26.7	33.4
kWh/kg PRODUCED	0.019	

STEAM CONSUMPTION (kg/h)

LOCATION	NORMAL USAGE	SIZING INFO
into product	84.0	112.0
extruder jackets	7.4	22.1
dryer	476.0	634.7
feed tank	25.0	40.0
TOTAL	592.4	808.7
kg/kg PRODUCED	0.423	

WATER CONSUMPTION (l/h)

LOCATION	NORMAL USAGE	SIZING INFO
into product	140.0	280.0
extruder jackets	928.0	1392.0
TOTAL	1068.0	1672.0
l/kg PRODUCED	0.763	

AIR CONSUMPTION (Nl/h)

WENGER

WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG REV: A
04/03/86 DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175 DENSITY (G/L): 400
NUMBER OF HEADS: 6 MAXIMUM CAPACITY (KG/HR): 1400

AIR CONSUMPTION (Nl/h)

LOCATION	NORMAL USAGE	SIZING INFO
dryer/cooler	920.4	1840.8
product spreader	30.0	60.0
TOTAL	950.4	1900.8
Nl/kg PRODUCED	0.679	

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         WENGER UTILITY CONSUMPTION AND SIZING INFORMATION

FOR: TEXAS A&M UNIVERSITY
PREPARED BY: MSG                                           REV: A
04/03/86      DETOXIFICATION OF DEFAT CASTOR-BEAN MEAL
X-175                                        DENSITY (G/L): 400
NUMBER OF HEADS: 6                   MAXIMUM CAPACITY (KG/HR): 1400
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  THESE UTILITY CONSUMPTION FIGURES WERE CALCULATED BASED ON THE
  FOLLOWING ASSUMPTIONS:

                 % STEAM INTO PRODUCT: 6.0
                 2 EXTRUDER JACKETS USING STEAM
                 % MOISTURE OUT OF EXTRUDER: 25.0
                 % MOISTURE OF PRODUCT INTO DRYER: 25
                 % MOISTURE OF PRODUCT OUT OF DRYER: 8
                 % WATER INTO PRODUCT: 10
                 0 STEAM FLOW METERS
                 0 PRESSURIZED PANELS

                 SYSTEM UTILITY COST PER TON ANALYSIS

                 EXTRUDER ELECTRICAL          $    5.46
                 DRYER/COOLER ELECTRICAL      $    1.34
                 STEAM                        $    8.46
                 WATER                        $    0.38
                 AIR                          $    0.01
                 TOTAL COST PER TON           $   15.65

                 SYSTEM ENERGY COST ASSUMPTIONS

                 $    0.070 PER KILOWATT
                 $   20.000 PER MT STEAM
                 $    0.500 PER CUBIC METER WATER
                 $    0.150 PER CUBIC METER GAS
                 $    0.014 PER CUBIC METER AIR
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WENGER